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Penoxsulam—Structure–activity relationships of triazolopyrimidine sulfonamides

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ABSTRACT

The discovery of the sulfonamide herbicides, which inhibit the enzyme acetolactate synthase (ALS), has resulted in many investigations to exploit their herbicidal activity. One area which proved particularly productive was the *N*-aryltriazolo[1,5-*c*]pyrimidine sulfonamides, providing three commercial herbicides, cloransulam-methyl, diclosulam and florasulam. Additional structure–activity investigations by reversing the sulfonamide linkage resulted in the discovery of triazolopyrimidine sulfonamides with cereal crop selectivity and high levels of grass and broadleaf weed control. Research efforts to exploit these high levels of weed activity ultimately led to the discovery of penoxsulam, a new herbicide developed for grass, sedge and broadleaf weed control in rice. Synthetic efforts and structure–activity relationships leading to the discovery of penoxsulam will be discussed.

1. Introduction

The triazolopyrimidine sulfonamide class of herbicides are the result of research efforts aimed at preparing bioisosteres of the sulfonyl urea herbicides.[1] Initial efforts focused on the *N*-(triazolo[1,5-*a*]pyrimidine) sulfonamides (**1**). Further investigations showed that reversing the sulfonamide linkage (–SO_2NH–) to give triazolo[1,5-*a*]pyrimidine sulfonanilides led to compounds with improved herbicidal activity and eventually to the discovery of flumetsulam (**2**).[2] The triazolopyrimidine sulfonamides have demonstrated competition with the amino acid leucine for binding to acetolactate synthase (ALS) isolated from cotton (*Gossypium hirsutum*).[3]

Since the discovery of flumetsulam, which was developed for broadleaf weed control in maize and soybeans, many investigations were initiated to examine the effects of sulfonanilides containing fused bicyclic heterocycles, and much of that work has been reviewed.[4,5] One area which proved to be quite productive was the triazolo[1,5-*c*]pyrimidine class of sulfonanilides. Investigation of this class led to the discovery of florasulam (**3**), which was commercialized for broadleaf weed control in cereals. All of the triazolopyrimidines commercialized at that point in time were developed for broadleaf weed control and all lacked commercial levels of grass weed control.

Subsequent research focused on the discovery of related sulfonamide analogs with a spectrum of activity that included activity on grass and broadleaf weeds with grass crop selectivity that was comparable to, or better than **2** or **3**. One initiative to expand the structure–activity relationship (SAR) around **3** and related analogs focused on reversing the sulfonamide linkage back to its original order.[6] This modification afforded grass crop selective sulfonamides which exhibited high levels of activity on both grass and broadleaf weeds. Additional investigations eventually led to the discovery of penoxsulam (**4**), which exhibits activity on both grass and broadleaf weeds with utility primarily in rice (Fig. 1).

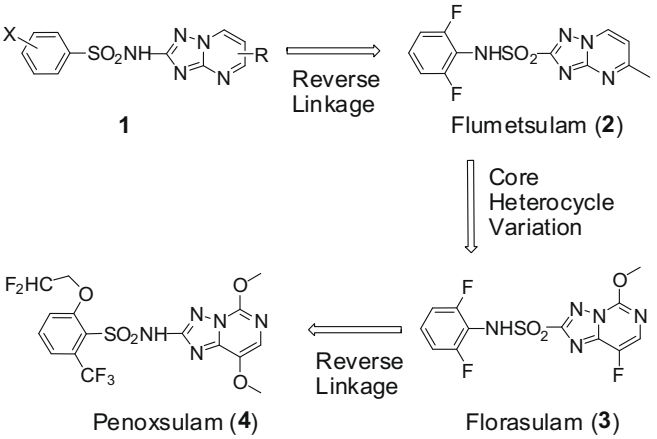

Figure 1. Evolution of triazolopyrimidine herbicides leading to Penoxsualm.

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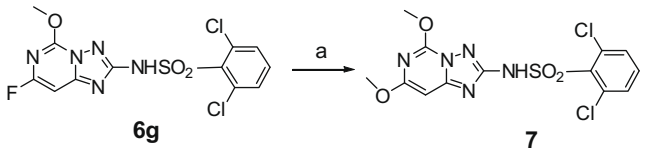
2. Results and discussion

2.1. Synthetic chemistry

The majority of the target triazolo[1,5-*c*]pyrimidine sulfonamides, such as **4**, were prepared via coupling 2-aminotriazolo[1,5-*c*]pyrimidines **5** with substituted benzenesulfonyl chlorides (Scheme 1).[7–9] In these transformations, pyridine and catalytic dimethylsulfoxide (DMSO) were essential for the formation of **6**. It is believed that the reactive sulfilimine intermediate was generated in situ, which allows for relatively non-nucleophilic aminotriazolopyrimidines to react with sulfonyl chlorides under mild conditions.[10–12] In addition to the coupling route, some sulfonamides were prepared through further derivatization of coupled targets as illustrated by the treatment of the 7-fluoro analog **6g** with sodium methoxide to afford the 7-methoxy analog **7** (Scheme 2). The substituted 2-aminotriazolo[1,5-*c*]pyrimidines **5** were prepared starting from 4-hydrazino-2-methylthiopyrimidines **8** (Scheme 3).[13,14] Compounds **8** were reacted with cyanogen bromide to give the 3-amino-5-methylthiotriazolo[4,3-*c*]pyrimidines **9**, usually as the hydrogen bromide salts. When treated with sodium methoxide, **9** undergoes a Dimroth rearrangement with loss of the methylthio group to form the desired 2-amino-5-methoxytriazolo[1,5-*c*]pyrimidines **5**. In this reaction, the Michael acceptor ethyl acrylate was used to consume the mercaptide by-product.

The arylsulfonyl chlorides used for the coupling reactions were prepared via directed ortho metalation as outlined in Schemes 4 and 5. When **10** was submitted to standard metalation conditions a considerable amount of the undesired 1,2,5-trisubstituted by-product was formed. However, when the lithiation was conducted using conditions of thermodynamic control the desired substitution pattern was obtained leading to **11** as the only product. Thus, when **10** was treated with a catalytic amount of diisopropylamine and less than 1 equiv of *n*-butyllithium, followed by equilibration and treatment with propyl disulfide, **11** was isolated in >99% yield (Scheme 4).[15] For sulfides **11** in which R is methoxymethyl (MOM), standard MOM deprotection conditions afforded the corresponding phenol (**12**), which was subsequently alkylated to give the appropriately substituted sulfide (**13**). Conversion of the sulfides (**11**, **13**) to their corresponding sulfonyl chlorides **14** was carried out using chloro-oxidation conditions, such as chlorine gas in aqueous acetic acid. It is noteworthy however, that attempts to prepare dialkoxybenzenesulfonyl chlorides using the described chloro-oxidation conditions resulted in ring chlorination prior to sulfonyl chloride formation. In these reactions, standard metalation conditions afford the desired regiochemistry and were therefore amenable to preparation via a simplified route. Thus, 1,3-dimethoxybenzene **15** was sequentially treated with *n*-butyllithium and sulfur dioxide to furnish the lithium benzenesulfinate.[16–18] Subsequent oxidation of the sulfinate with sulfuryl chloride afforded 2,6-dimethoxybenzenesulfonyl chloride **16** (Scheme 5) in good yield. In most cases the sulfonyl chlorides were reacted with the aminotriazolo[1,5-*c*]pyrimidine immediately after workup without further purification.

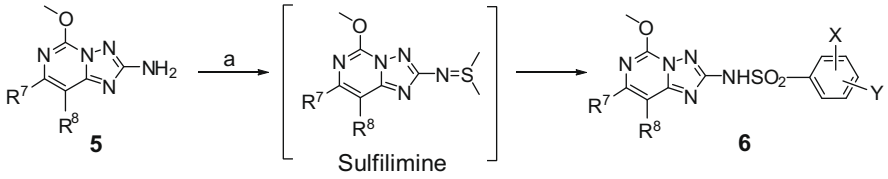


Scheme 2. Reagents: NaOMe, MeOH.

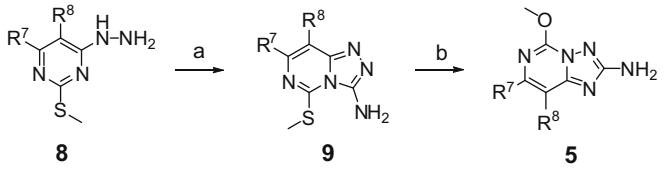
2.2. SAR development

Initial efforts to determine the SAR of the triazolo[1,5-*c*]pyrimidines (**6**) focused on substitutions in the 7- and 8-positions of **6**. Extensive SAR work around florasulam (**3**) showed the highest levels of activity were obtained with a methoxy in the 5-position, and that varying substitution in the 7- and 8-positions resulted in different levels of activity on weeds and crops. Thus, analogs of **6** were prepared with a methoxy in the 5-position. A summary of the herbicidal activity for compounds substituted in the 7- and 8-positions of **6** (R^7 and R^8, respectively, Fig. 2) is given in Table 1. In general compounds with substitution in the 8-position have higher levels of activity than those with substitutions in the 7-position. Weak levels of activity are observed with no substitution in either the 7- or 8-positions (**6a**). Both the 8-methoxy analog **6b** and 8-chloro analog **6c** have good levels of activity on both broadleaf and grass weeds. Compared to **6c** the 8-fluoro analog **6d** causes a decrease in activity. The 8-methyl analog **6e**, like **6d**, has much less activity on grass and broadleaf weeds than either **6b** or **6c**. Increasing the 8-alkoxy size from methoxy **6b** to ethoxy **6f** causes a decrease in activity on both broadleaf and grass weeds. The 7-methoxy analog **7**, unlike the 8-methoxy analog **6b**, had very weak activity on both grass and broadleaf weeds. The trends observed for in vivo activity correlate with the trends observed for in vitro activity. The most active compounds, **6b** and **6c**, on grass and broadleaf weeds also cause significant injury to rice (*Oryza sativa*).

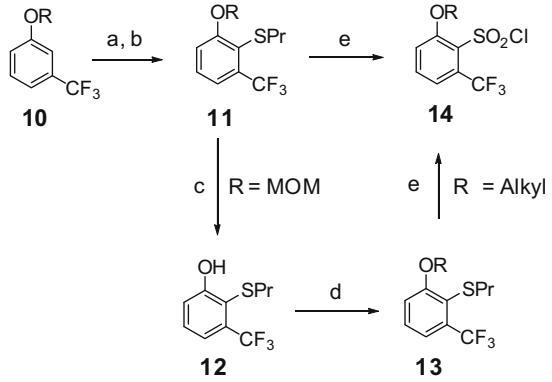
Further SAR development focused on substitutions about the phenyl ring. In these studies the highly active 5,8-dimethoxy substitution pattern on the triazolo[1,5-*c*]pyrimidine ring was retained. The in vivo activity for compounds with various substitutions on the phenyl ring (Fig. 3) is shown in Table 2. In general, both mono and disubstitutions can impart good levels of activity on both broadleaf and grass weeds. For mono substitutions the methoxy analog **6h** showed better activity on both grass and broadleaf weeds than a trifluoromethyl **6i**. By comparing the dichloro substituted sulfonamides **6b**, **6j** and **6k** it can be seen that 2,6-disubstitution imparts the best activity on both grass and broadleaf weeds. For disubstitutions, when one of the substituents was methoxy good levels of activity are obtained when the 6-position was substituted with either an electron donating substituent (**6l**, 6-OMe) or an electron withdrawing substitutent (**6m**, 6-CF_3). Many of the highly active compounds were also very injurious to rice. It was observed that increasing the size of the alkoxy from methoxy to ethoxy and keeping a trifluoromethyl in the 6-position (**6n**) resulted in loss of activity on most grass weeds. However, **6n** showed less injury to rice while maintaining good levels of activity



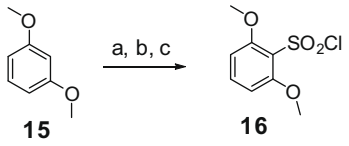
Scheme 1. Reagents: $ArSO_2Cl$, pyridine, DMSO(catalytic), CH_3CN.



Scheme 3. Reagents: (a) BrCN, *i*-PrOH; (b) NaOMe, MeOH, ethyl acrylate.



Scheme 4. Reagents: (a) BuLi (0.95 equiv), TMEDA, $(i\text{-pr})_2NH$ (10 mol %), THF or Et_2O; (b) $(PrS)_2$; HCl, MeOH; (d) phenol alkylation; (e) Cl_2, H_2O, HOAc.



Scheme 5. Reagents: (a) BuLi (1.2 equiv), TMEDA, THF or Et_2O; (b) SO_2, Et_2O; (c) SO_2Cl_2, hexane.

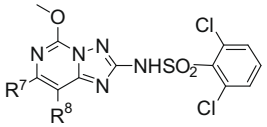


Figure 2. Substitutions in the 7- and 8-positions of the triazolopyrimidine ring.

on broadleaf weeds and barnyard grass (*Echinochloa crus-galli*). When the ethoxy group was substituted with terminal fluorine (**6o**) the activity on broadleaf weeds was maintained and there was slightly better activity on barnyard grass than was observed for **6n**. The 2-fluoropropoxy analog **6p** shows a significant decrease in activity on barnyard grass compared to either **6n** or **6o**.

Table 1
Dependence of herbicidal activity on substitutions in the 7- and 8-positions of the triazolopyrimidine ring (Fig. 2)

Compd	R^7	R^8	BW GR_{80} (ppm)	GW GR_{80} (ppm)	*Oryza sativa* GR_{20} (ppm)	ALS I_{50} (nM)
6a	H	H	475	>2000	500	1040
6b	H	OMe	2	10	8	0.6
6c	H	Cl	1	15	4	0.6
6d	H	F	19	309	>125	12
6e	H	Me	<15	>250	250	7
6f	H	OEt	216	>500	250	2
6g	F	H	62	574	8	27
7	OMe	H	>1000	>1000	>1000	195

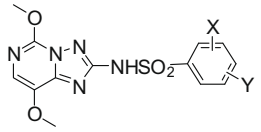


Figure 3. Substitutions on the phenyl ring of the triazolopyrimidine sulfonamides.

Table 2
Herbicidal activity for various mono- and disubstituted phenyl analogs (Fig. 3)

Compd	X	Y	BW GR_{80} (ppm)	GW GR_{80} (ppm)	*Oryza sativa* GR_{20} (ppm)	*Echinochloa crus-galli* GR_{80} (ppm)
6h	2-OMe	H	1	1	<1	2
6i	2-CF_3	H	13	435	<1	250
6b	2-Cl	6-Cl	2	10	8	4
6j	2-Cl	5-Cl	19	>1000	<2	1000
6k	3-Cl	5-Cl	126	>1000	62	>1000
6l	2-OMe	6-OMe	0.5	<0.1	<0.5	<0.5
6m	2-OMe	6-CF_3	<0.2	1	2	1
6n	2-OEt	6-CF_3	2	191	250	4
6o	2-$O(CH_2)_2F$	6-CF_3	6	66	250	1
6p	$O(CH_2)_3F$	6-CF_3	15	250	>250	125

Based on the findings shown in Table 2, further investigations were initiated into 2-fluoroalkoxy-6-trifluoromethyl substituted phenyl analogs. Table 3 summarizes the activity on rice and key rice weeds for specific 2-alkoxy-6-trifluoromethylphenyl substituted analogs (Fig. 4) when applied as a water-injected treatment in the greenhouse to rice and weeds in the one to three leaf stage. In these greenhouse trials, rates were expressed as gram active ingredient per hectare (g ai/ha). In this test the ethoxy analog **6n** caused significant injury to rice. The 2-fluoroethoxy analog **6o** showed slightly less injury to rice with good control of key weeds. The trifluoroethoxy analog **6q** shows even less injury to rice with some loss in weed control activity compared to **6o** or **6n**. The difluoroethoxy analog **6r** shows less injury to rice than **6q** and better activity on the key weeds. The difluoroisopropoxy analog **6s** demonstrated the least injury to rice while maintaining activity on key weeds comparable to **6r**. Based on these results **6o**, **6r** and **6s** were selected for further field testing in key rice countries.

2.3. Field results—Screening

Field testing of the three alkoxy trifluoromethylphenyl analogs **6o**, **6r** and **6s** under different rice growing conditions in several rice growing countries quickly sorted the analogs in order of performance for weed control and rice. Table 4 summarizes the rice tolerance and weed control activity of the analogs when applied as water injected treatments to transplanted japonica rice and weeds at the three leaf stage under field conditions. These results demonstrate the poor rice selectivity of **6o** as compared to **6r** and **6s**, but better weed control than **6s** and slightly better weed control compared to **6r**. Based on rice selectivity and weed control, **6r** was chosen as the best analog in this study. Also, analog **6r** appeared to have significantly longer residual weed control activity than **6s**, which is an important criterion for a rice herbicide (data not shown).

Table 5 shows the results of testing the three analogs in direct seeded indica rice as foliar applied treatments to rice and weeds at the 2–3 leaf stage. Early greenhouse results had demonstrated that indica rice had much better tolerance to this chemistry than japonica rice. The results shown here demonstrated the excellent indica rice tolerance to **6o**, **6r** and **6s**. From a weed control perspective **6o** had the highest level of weed control activity, **6s** had the lowest level of weed control activity, and **6r** was intermediate

Table 3
Herbicidal activity of alkoxy trifluoromethyl phenyl analogs (Fig. 4) when applied as a water injection treatment on transplanted paddy rice and selected weeds

Compd	Y	*Oryza sativa* GR_{20} (g ai/ha)	*Echinochloa crus-galli* GR_{80} (g ai/ha)	*Monochoria vaginalis* GR_{80} (g ai/ha)	*Scirpus juncoides* GR_{80} (g ai/ha)	*Cyperus difformis* GR_{80} (g ai/ha)
6n	OEt	1	1	1	3	N A
6o	$O(CH_2)_2F$	14	10	5.2	9	8
6q	OCH_2CF_3	51	19	5	21	36
6r	OCH_2CHF_2	75	12	<2	12	14
6s	$OCH(CH_2F)_2$	140	14	1	9	31

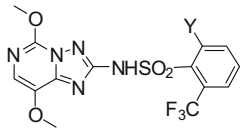


Figure 4. 2-Alkoxy-6-trifluoromethylphenyltriazolo [1,5-*c*]pyrimidine sulfonamide analogs.

but close to **6o**. Ratings at 30 and 60 days demonstrated the poor residual weed control activity of **6s** compared to **6o** and **6r** (data not shown).

Table 6 shows results from testing **6o** and **6r** as post-emergence foliar applications in direct seeded japonica rice at the 3–4 leaf stage. The results in japonica rice were very compelling and quickly showed the advantage of **6r** over **6o** from a rice selectivity standpoint. The level of rice injury from **6o** was not acceptable on japonica rice at rates necessary for commercial levels of weed control.

2.4. Field results—Global field trials

Based on the poor selectivity of **6o** in japonica direct seeded rice, as well as, the better foliar weed control and longer soil residual activity of **6r** compared to **6s**, **6r** was chosen for development as a rice herbicide with the common name 'penoxsulam'.

From 1998 to the end of 2007, over 2000 rice field trials have been conducted testing **6r** (penoxsulam) alone and in tank mixes with many other products and multiple formulations. Penoxsulam has been shown to provide effective weed control as a post-emergence foliar application in direct-seeded rice globally;[19–21] as a foliar application in water-seeded japonica rice in Europe and Eufrasia;[22] as a post-emergence foliar application in Vietnam and China[23] and as a water-injected application into flooded paddy rice in Korea.[24]

As of 2008, penoxsulam has been registered as a rice herbicide in 28 countries, being used for selective weed control in transplanted rice, direct-seeded japonica and indica rice, and in water-seeded japonica and indica rice.

Table 7 is a global summary of weed control performance as determined by many field trial evaluations of multiple rates of penoxsulam under local rice growing conditions around the world in many countries. We have seen that there are some biotype differences within a weed species across geographies, so the following table serves as a summary and guide to penoxsulam weed control performance.

Table 4
Herbicidal activity of specific alkoxy trifluoromethyl phenyl analogs (Fig. 4) when applied as a water injection treatment on transplanted paddy rice and selected weeds

Compd	Y	*Oryza sativa* GR_{10} (g ai/ha)	*Echinochloa crus-galli* GR_{80} (g ai/ha)	*Monochoria vaginalis* GR_{80} (g ai/ha)	*Scirpus juncoides* GR_{80} (g ai/ha)
6o	$O(CH_2)_2F$	8	19	4.4	5
6r	OCH_2CHF_2	35	26	6	9
6s	$OCH(CH_2F)_2$	70	35	12	15

Table 5
Herbicidal activity of alkoxy trifluoromethyl phenyl analogs (Fig. 4) when applied as post-emergence foliar treatment to direct seeded indica rice and selected weeds

Compd	Y	*Oryza sativa* GR_{10} (g ai/ha)	*Echinochloa crus-galli* GR_{80} (g ai/ha)	*Scirpus juncoides* GR_{80} (g ai/ha)	*Cyperus difformis* GR_{80v}
6o	$O(CH_2)_2F$	>70	5	4	4
6r	OCH_2CHF_2	>70	7	6	7
6s	$OCH(CH_2F)_2$	>70	15	20	12

Table 6
Herbicidal activity of alkoxy trifluoromethyl phenyl analogs (Fig. 4) when applied as a post-emergence foliar treatment to direct seeded japonica rice and selected weeds

Compd	Y	*Oryza sativa* GR_{10} (g ai/ha)	*Echinochloa crus-galli* GR_{80} (g ai/ha)	*Sesbania exaltata* GR_{80} (g ai/ha)
6o	$O(CH_2)_2F$	12	12	10
6r	OCH_2CF_2H	65	9	7

3. Materials and methods

3.1. Synthesis

All melting points were recorded on a Thomas Hoover melting point apparatus using the appropriate partial immersion thermometer and are uncorrected. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were obtained in either $CDCl_3$ or DMSO-d_6, used as purchased, and were recorded on a Varian Gemini spectrometer and referenced to an internal standard of tetramethylsilane (TMS ^{1}H: δ 0.00). ^{1}H–^{1}H couplings are assumed to be first order and peak multiplicity is reported as s (singlet), d (doublet), t (triplet), q (quartet), sext (sextet), m (multiplet), or br (broad). Combustion analyses were performed either in house or by Midwest Microlab, Indianapolis, IN. Mass spectral data were obtained on a Hewlett-Packard series 1100 mass selective detector, Hewlett-Packard 5890 series II with a Hewlett-Packard 5890A mass spectrometer, or a Hewlett-Packard 6890 series GC system with a 5973 mass selective detector. Flash or gravity chromatography was performed using 230–400 mesh silica gel from EM Science, Darmstadt, Germany using reagent grade solvents. Reaction were monitored by thin layer chromatography (TLC) on 250 μm precoated Analtech Uniplate plates and visualized will ultraviolet light. Unless otherwise noted, materials were obtained from commercial suppliers and used as is.

Compounds **6a** and **6c–s** were prepared according to the procedure for **6b** below:

3.1.1. 2,6-Dichloro-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzene sulfonamide (6b)

A round bottom flask fitted with a drying tube ($CaSO_4$) was charged with 2-amino-5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimi-

Table 7
Global weed control performance summary of penoxsulam on rice weeds susceptible, moderately susceptible and tolerant are an indication of weed susceptibility to applications of penoxsulam in rice on these plant species at local use rates across different geographies

Susceptible		Moderately susceptible	Tolerant
Acalypha australis	*Heteranthera limosa*	*Butomus umbellatus*	*Aneilema keisak*
Aeschynomene spp.	*Kyllinga squamulate*	*Commelina benghalensis*	*Brachiaria* spp.
Alisma plantago-aquatica	*Lindernia* spp.	*Corchorus* spp.	*Cenchrus echinatus*
Alternanthera philoxeroides	*Lythrum junceum*	*Cyperus esculentus*	*Dactyloctenium* spp.
Amaranthus spp.	*Malachra alceifolia*	*Cyperus rotundus*	*Digitaria* spp.
Ammannia spp.	*Melochia* spp.	*Cyperus serotinus*	*Eleusine indica*
Bacopa spp.	*Monochoria* spp.	*Eriochloa* spp.	*Eriochloa punctata*
Baltimora recta	*Nasturtium officinale*	*Erucaria* spp.	*Euphorbia heterophylla*
Bergia capensis	*Nymphaea* spp.	*Euphorbia nutans*	*Leptochloa* spp.
Bidens tripartita	*Oenanthe javanica*	*Glyceria declinata*	*Murdannia nudiflora*
Boerhaavia diffusa	*Phyllanthus niruri*	*Hyssopus* spp.	*Oryza sativa*
Caperonia spp.	*Physalis angulata*	*Ipomoea* spp.	*Panicum* spp.
Cardamine parviflora	*Polygonum* spp.	*Ischaemum rugosum*	*Paspalum* spp.
Chenopodium spp.	*Portulaca oleracea*	*Jussiaea abyssinica*	*Rottboellia* spp.
Cleome viscosa	*Rotala indica*	*Ludwigia* spp.	*Scirpus planiculmis*
Commelina communis	*Sagittaria* spp.	*Marsilea* spp.	*Setaria* spp.
Cyperus spp (annual)	*Scirpus juncoides*	*Potamogeton distinctum*	
Echinochloa spp. (annual)	*Scirpus mucronatus*	*Scirpus maritimus*	
Echinochloa polystachya	*Scleria pterota*	*Sida* spp.	
Eclipta alba	*Sesbania exaltata*	*Spigelia anthelmia*	
Elatine trianda	*Sphenoclea zeylanica*		
Eleocharis spp.	*Spilanthes acmella*		
Fimbristylis spp.	*Tridax procumbens*		
	Xanthium strumarium		

dine (**5a**, 1.0 g, 5.1 mmol), 2,6-dichlorobenzenesulfonyl chloride (1.26 g, 5.1 mmol), and anhydrous CH_3CN (15 mL). To this mixture was added anhydrous pyridine (0.82 mL, 10.2 mmol) and anhydrous DMSO (72 μL, 1.0 mmol). After 6 h, HPLC analysis of the reaction mixture indicated full consumption of the amine. The CH_3CN was removed in vacuo and the residue dissolved in CH_2Cl_2 (200 mL). The organic layer was washed with 2 N HCl (100 mL), H_2O (2× 100 mL), dried ($MgSO_4$), filtered and evaporated in vacuo to give a tan powder. The tan powder was stirred with Et_2O (100 mL) for 18 h. The solids were filtered, rinsed with Et_2O, and dried in a vacuum oven (50 °C, high vacuum) to afford **6b** as a tan powder (1.30 g, 63% yield): mp 219–221 °C; ^{1}H NMR (DMSO-d_6) δ 12.7 (br s, 1H), 7.66–7.52 (m, 4H), 4.11 (s, 3H), 3.88 (s, 3H). Anal. Calcd for $C_{13}H_{11}Cl_2N_5O_4S$: C, 38.63; H, 2.74; N, 17.33; S, 7.93. Found: C, 38.57; H, 2.81; N, 17.35; S, 8.13. In some cases it was necessary to further purify the product by column chromatography (SiO_2, CH_2Cl_2/EtOH, 95:5).

3.1.2. 2,6-Dichloro-*N*-(5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6a)

The product was isolated as a tan solid (14% yield): mp 225–226 °C; ^{1}H NMR (DMSO-d_6) δ 12.8 (br s, 1H), 7.99 (d, J = 6.2 Hz, 1H), 7.68–7.56 (m, 3H), 7.26 (d, J = 6.3 Hz, 1H), 4.17 (s, 3H).

3.1.3. 2,6-Dichloro-*N*-(8-chloro-5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6c)

The product was isolated as a white solid (61% yield): mp 214–216 °C (d); ^{1}H NMR (DMSO-d_6) δ 12.9 (br s, 1H), 8.10 (s, 1H), 7.67–7.58 (m, 3H), 4.13 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 158.8, 151.9, 148.6, 142.7, 135.2, 134.9, 134.4, 132.1, 110.4, 57.0; MS-DIP (CI) m/z 408 ([M]$^+$). Anal. Calcd for $C_{12}H_8Cl_3N_5O_3S$: C, 35.27; H, 1.97; N, 17.14; S, 7.85. Found: C, 35.27; H, 1.93; N, 16.84; S, 7.47.

3.1.4. 2,6-Dichloro-*N*-(8-fluoro-5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6d)

The product was isolated as a tan solid (50% yield): mp 211–212 °C; ^{1}H NMR (DMSO-d_6) δ 8.07 (d, J = 0.9 Hz, 1H), 7.52–7.65 (m, 3H), 4.10 (s, 3H). Anal. Calcd for $C_{12}H_8Cl_2N_5O_3S$: C, 36.75; H, 2.06; N, 17.86; S, 8.18. Found: C, 36.77; H, 2.08; N, 17.72; S, 8.03.

3.1.5. 2,6-Dichloro-*N*-(5-methoxy-8-methyl-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6e)

The product was isolated as a white solid (86% yield): mp 218–220 °C (d); ^{1}H NMR (DMSO-d_6) δ 12.7 (br s, 1H), 7.77 (s, 1H), 7.66–7.55 (m, 3H), 4.09 (s, 3H), 2.21 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 158.2, 154.1, 147.6, 142.0, 134.8, 134.7, 133.9, 131.6, 113.0, 55.9, 12.2; MS-DIP (CI) m/z 388 ([M]$^+$).

3.1.6. 2,6-Dichloro-*N*-(8-ethoxy-5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6f)

The product was isolated as a white solid (36% yield): mp 217–218 °C; ^{1}H NMR (DMSO-d_6) δ 12.8 (br s, 1H), 7.67–7.54 (m, 4H), 4.16 (q, J = 7.0 Hz, 2H), 4.07 (s, 3H), 1.30 (t, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 157.9, 148.4, 144.0, 138.0, 134.4, 133.8, 131.6, 126.3, 98.0, 65.8, 55.8, 14.5; MS-DIP (EI) m/z 417 ([M]$^+$). Anal. Calcd for $C_{14}H_{13}Cl_2N_5O_4S$: C, 40.20; H, 3.13; N, 16.74; S, 7.67. Found: C, 40.10; H, 3.13; N, 16.78; S, 7.48.

3.1.7. 2,6-Dichloro-*N*-(7-fluoro-5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6g)

The product was isolated as a tan powder (93% yield): mp 216–218 °C; ^{1}H NMR (DMSO-d_6) δ 12.82 (br s, 1H), 7.67–7.57 (m, 3H), 6.99 (s, 1H), 4.14 (s, 3H); ^{13}C (DMSO-d_6) δ 162.6, 160.2, 159.4, 156.1, 156.0, 148.7, 148.5, 134.7, 134.3, 133.9, 131.6, 85.0, 84.6, 57. 57.0. Anal. Calcd for $C_{12}H_8Cl_2FN_5O_3S$: C, 36.75; H, 2.06; N, 17.86; S, 8.17. Found: C, 36.93; H, 1.88; N, 17.92; S, 8.32.

3.1.8. 2-Methoxy-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6h)

The product was isolated as a tan solid (90% yield): mp 231–233 °C; ^{1}H NMR (DMSO-d_6) δ 11.8 (br s, 1H), 7.87 (dd, J = 1.5, 7.9 Hz, 1H), 7.57–7.54 (m, 2H), 7.15 (d, J = 8.2 Hz, 1H), 7.07 (t, J = 7.6 Hz, 1H), 4.03 (s, 3H), 3.85 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 158.4, 156.6, 148.0, 143.7, 139.3, 135.1, 130.6, 127.1, 123.4, 119.9, 112.9, 57.0, 56.1, 55.7. Anal. Calcd for

$C_{14}H_{15}N_5O_5S$: C, 46.02; H, 4.14; N, 19.17; S 8.77. Found: C, 46.17; H, 4.00; N, 19.00; S, 8.99.

3.1.9. 2-Trifluoromethyl-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6i)

The product was isolated as a white powder (20% yield): mp 189–191 °C; ^{1}H NMR (DMSO-d_6) δ 12.4 (br s, 1H), 8.32 (d, J = 7.3 Hz, 1H), 8.00 (d, J = 7.9 Hz, 1H), 7.91–7.86 (m, 2H), 7.61 (s, 1 H), 4.07 (s, 3H), 3.88 (s, 3H).

3.1.10. 2,5-Dichloro-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6j)

The product was isolated as a white solid (62% yield): mp 219–221 °C; ^{1}H NMR (DMSO-d_6) δ 12.70 (br s, 1H), 8.16 (s, 1H), 7.79–7.60 (m, 3H), 4.08 (s, 3H), 3.90 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 157.6, 148.1, 143.8, 139.3, 138.4, 134.5, 133.4, 131.8, 131.6, 129.6, 124.3, 57.2, 55.8; MS-DIP (CI) m/z 404 ([M]$^+$). Anal. Calcd for $C_{13}H_{11}Cl_2N_5O_4S$: C, 38.63; H, 2.74; N, 17.33; S, 7.93. Found: C, 38.60; H, 2.85; N, 17.35; S, 8.13.

3.1.11. 3,5-Dichloro-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6k)

The product was isolated as a white solid (34% yield): mp 195–197 °C; ^{1}H NMR (DMSO-d_6) δ 12.80 (br s, 1H), 8.01 (s, 1H), 7.99 (s, 2H), 7.65 (s, 1H), 4.12 (s, 3H), 3.93 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 157.9, 148.1, 144.0, 142.7, 139.4, 134.8, 133.0, 125.9, 124.2, 57.3, 55.9; MS-DIP (CI) m/z 404 ([M]$^+$). Anal. Calcd for $C_{13}H_{11}Cl_2N_5O_4S$: C, 38.63; H, 2.74; N, 17.33; S, 7.93. Found: C, 38.80; H, 2.68; N, 17.09; S, 7.78.

3.1.12. 2,6-Dimethoxy-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)-benzenesulfonamide (6l)

The product was isolated as an off-white solid (68% yield): mp 239–240 °C; ^{1}H NMR (DMSO-d_6) δ 11.54 (s, 1H), 7.55 (s, 1H), 7.44 (t, J = 8.4 Hz, 1H), 6.74 (d, J = 8.4 Hz, 2H), 4.06 (s, 3H), 3.88 (s, 3H), 3.76 (s, 6H); ^{13}C NMR (DMSO-d_6) δ 159.2, 158.4, 148.0, 143.7, 139.3, 134.1, 123.3, 117.3, 105.5, 57.0, 56.6, 55.7. Anal. Calcd for $C_{15}H_{17}N_5O_6S$: C, 45.57; H, 4.33; N, 17.71; S, 8.11. Found: C, 45.47; H, 4.34; N, 17.54; S, 8.09.

3.1.13. 2-Methoxy-6-trifluoromethyl-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)-benzenesulfonamide (6m)

The product was isolated as a white solid (29% yield): mp 238–240 °C; ^{1}H NMR (DMSO-d_6) δ 11.87 (s, 1H), 7.74 (t, J = 8.1, 8.4 Hz, 1H), 7.56–7.51 (m, 3H), 4.05 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 158.3, 158.0, 148.1, 143.8, 139.3, 134.2, 129.6, 129.3, 129.0, 126.7, 126.8, 124.2, 123.9, 121.4, 119.54, 119.47, 118.2, 57.2, 55.8; MS-DIP (CI) m/z 434 ([M+1]$^+$). Anal. Calcd for $C_{15}H_{14}F_3N_5O_5S$: C, 41.57; H, 3.26; N, 16.16; S, 7.40. Found: C, 41.46; H 3.20; N, 16.26; S, 7.34.

3.1.14. 2-Ethoxy-6-trifluoromethyl-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)-benzenesulfonamide (6n)

The product was isolated as a white solid (29% yield): mp 232–234 °C (d); ^{1}H NMR (DMSO-d_6), δ 11.8 (br s, 1H), 7.72 (t, J = 8.3 Hz, 1H), 7.59 (s, 1H), 7.53 (d, J = 8.0 Hz, 2H), 4.23 (q, J = 7.0 Hz, 2H), 4.06 (s, 3H), 3.86 (s, 3H), 1.20 (t, J = 7.0 Hz, 3H); LC–MS (ESI) m/z 446 ([M−H]$^-$). Anal. Calcd for $C_{16}H_{16}F_3N_5O_5S$: C, 42.96; H, 3.60; N, 15.65; S, 7.17. Found: C, 42.85; H, 3.51; N, 15.71; S, 7.24.

3.1.15. 2-(2-Fluoroethoxy)-6-trifluoromethyl-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6o)

The product was isolated as a white solid (52% yield): mp 233–235 °C; ^{1}H NMR (DMSO-d_6) δ 11.82 (br s, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.58–7.54 (m, 3H), 4.85–4.69 (dt, J = 3.8, 47.7 Hz, 2H), 4.44–4.34 (dt, J = 3.8, 30.0 Hz, 2H), 4.02 (s, 3H), 3.82 (s, 3H); ^{13}C NMR (DMSO-d_6), δ 164.4, 158.3, 154.0, 147.9, 146.6, 146.1, 143.7, 141.5, 139.2, 131.1, 124.3, 123.9, 122.0, 118.3, 57.1, 55.8, 53.4; MS-DIP (CI) m/z 465 ([M]$^+$). Anal. Calcd for $C_{16}H_{15}F_4N_5O_5S$: C 41.29; H, 3.25; N, 15.05; S, 6.89. Found: C, 41.35; H, 3.09; N, 14.89; S, 6.94.

3.1.16. 2-(3-Fluoropropoxy)-6-trifluoromethyl-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6p)

The product was isolated as a tan solid (30% yield): mp 210–212 °C; ^{1}H NMR (DMSO-d_6) δ 11.79 (s, 1H), 7.72 (t, J = 8.1 Hz, 1H), 7.56–7.52 (m, 3H), 4.56 (dt, J = 5.8, 47.5 Hz, 2H), 4.19 (t, J = 5.8 Hz, 2H), 4.02 (s, 3H), 3.83 (s, 3H), 2.12 (dp, J = 5.8, 26.1 Hz, 2H); LC–MS (ESI) m/z 480 ([M+H]$^+$). Anal. Calcd for $C_{17}H_{17}F_4N_5O_5S$: C, 42.57; H, 3.57; N, 14.61; S, 6.69. Found: C, 42.48; H, 3.53; N, 14.42; S, 6.57.

3.1.17. 2-(2,2,2-Trifluoroethoxy)-6-trifluoromethyl-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6q)

The product was isolated as a white solid (30% yield): mp 211–213 °C; ^{1}H NMR (DMSO-d_6) δ 11.87 (s, 1H), 7.80 (t, J = 8.5 Hz, 1H), 7.67 (d, J = 7.9 Hz, 2H), 7.57 (s, 1H), 4.94 (q, J = 8.8 Hz, 2H), 4.04 (s, 3H), 3.83 (s, 3H); LC–MS (ESI) m/z 500 ([M−H]$^-$). Anal. Calcd for $C_{16}H_{13}F_6N_5O_5S$: C, 38.33; H, 2.61; N, 13.97; S, 6.39. Found: C, 38.26; H, 2.59; N, 13.89; S, 6.40.

3.1.18. 2-(2,2-Difluoroethoxy)-6-trifluoromethyl-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6r)

The product was isolated as a tan solid (24% yield): mp 223–224 °C; ^{1}H NMR (DMSO-d_6) δ 11.89 (s, 1H), 7.76 (t, J = 8.2 Hz, 1H), 7.62 (m, 3H), 6.50 (tt, J = 4.2, 54.9 Hz, 1H), 4.48 (td, J = 3.8, 13.7 Hz, 2H), 4.03 (s, 3H), 3.83 (s, 3H); LC–MS (ESI) m/z 482 ([M−H]$^-$). Anal. Calcd for $C_{16}H_{14}F_5N_5O_5S$: C, 39.76; H, 2.92; N, 14.49; S, 6.63. Found: C, 39.52; H, 2.74; N, 14.27; S, 6.82.

3.1.19. 1-[2-Fluoro-1-(fluoromethyl)ethoxy]-6-trifluoromethyl-*N*-(5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (6s)

The product was isolated as a white solid (19% yield): mp 229–231 °C; ^{1}H NMR (DMSO-d_6) δ 11.81 (br s, 1H), 7.79–7.69 (m, 2H), 7.61–7.59 (m, 2H), 5.42–5.23 (m, 1H), 4.84–4.55 (m, 4H), 4.06 (s, 3H), 3.85 (s, 3H); LC–MS (ESI) m/z 498 ([M−H]$^+$). Anal. Calcd for $C_{17}H_{16}F_5N_5O_5S$: C, 41.05; H, 3.24; N, 14.08; S, 6.45. Found: C, 40.95; H, 3.12; N, 13.96; S, 6.57.

3.1.20. 2,6-Dichloro-*N*-(5,7-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (7)

A round bottom flask was charged with 2,6-dichloro-*N*-(5-methoxy-7-fluoro-1,2,4-triazolo[1,5-*c*]pyrimidin-2-yl)benzenesulfonamide (**6g**, 0.50 g, 1.3 mmol) and anhydrous MeOH (3 mL). To this mixture was added a solution of sodium methoxide (0.61 mL, 2.7 mmol, 25% in MeOH) at which time all the solids dissolved giving a dark solution. After 2 h, a solid had precipitated from the solution. The mixture was stirred for 18 h, the reaction quenched with HOAc (1 mL), and the methanol removed in vacuo to give a tan solid. The crude material was purified by column chromatography (SiO_2, CH_2Cl_2/EtOH, 95:5) to afford **7** as a white powder (0.30 g, 56% yield): mp 245–247 °C; ^{1}H NMR (DMSO-d_6) δ 12.6 (br s, 1H), 7.65–7.57 (m, 3H), 6.45 (s, 1H), 4.11 (s, 3H), 3.88 (s, 3H). Anal. Calcd for $C_{13}H_{11}Cl_2N_5O_4S$: C, 38.63; H, 2.74; N, 17.33; S, 7.93. Found: C, 38.43; H, 2.75; N, 17.19; S, 8.09.

Compounds **5b–g** were prepared according to the procedure for **5a** below:

3.1.21. 2-Amino-5,8-dimethoxy-1,2,4-triazolo[1,5-*c*]pyrimidine (5a)

4-Hydrazino-5-methoxy-2-methylthiopyrimidine **8a** (5.0 g, 27 mmol) was mixed with isopropyl alcohol (IPA, 50 mL). To this mixture was added a solution of cyanogen bromide (10 mL, 30 mmol, 3 M, CH_2Cl_2). The mixture was stirred at room temperature for 30 min then warmed to 50 °C. After 1 h at 50 °C, there was no hydrazinopyrimidine detected by thin-layer chromatography (TLC, hexane/EtOAc, 1:1). The reaction mixture was cooled to room temperature and diluted with Et_2O (150 mL). The solids were filtered, washed with Et_2O, and dried in a vacuum oven (50 °C, high vacuum) to afford the crude 3-amino-8-methoxy-5-methylthio-1,2,4-triazolo[4,3-*c*]pyrimidine hydrobromide as a tan solid (**9a**, 7.1 g, 90% yield): mp 180–182 °C; ^{1}H NMR (DMSO-d_6) δ 7.6 (br s, 2H), 7.5 (s, 1H), 3.9 (s, 3H), 2.7 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 147.4, 141.8, 140.9, 120.3, 57.0, 14.2. The crude 3-amino-8-methoxy-5-methylthio-1,2,4-triazolo[4,3-*c*]pyrimidine hydrobromide (55.7 g, 191 mmol) and methyl acrylate (31 mL, 286 mmol) were mixed with CH_3OH (200 mL). The mixture was cooled in an ice bath. To the cold mixture was slowly added a solution of sodium methoxide (60 mL, 286 mmol, 25%) in CH_3OH. After all the sodium methoxide was added, the mixture was warmed to room temperature and stirred for 18 h. To the reaction mixture was added HOAc (5 mL) and the solids were collected by filtration. The solids were partitioned between Et_2O (200 mL) and H_2O (200 mL). The insoluble material was collected by filtration and dried in a vacuum oven (50 °C, high vacuum) to afford the crude product as a tan solid (26.8 g, 72% yield). A sample of the crude material (2.0 g) was recrystallized from DMF to afford **5a** as a tan solid (0.80 g): mp 201–203 °C; ^{1}H NMR (DMSO-d_6) δ 7.5 (s, 1H), 6.3 (s, 2H), 4.1 (s, 3H), 3.9 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.0, 148.5, 143.7, 138.6, 123.1, 57.0, 55.4. Anal. Calcd for $C_7H_9N_5O_2$: C, 43.08; H, 4.65; N, 35.88. Found: C, 43.24; H, 4.67; N, 35.59.

3.1.22. 2-Amino-5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidine (5b)

The intermediate 2-amino-5-methylthio-1,2,4-triazolo[4,3-*c*]pyrimidine (**9b**) was isolated as a pale yellow solid (0.65 g, 26% yield): mp 232–234 °C (d); ^{1}H NMR (DMSO-d_6) δ 7.52 (d, J = 6.6 Hz, 1H), 7.13 (d, J = 6.7 Hz, 1H), 6.08 (br s, 2H), 2.62 (s, 3H). The product (**5b**) was isolated as a tan powder (70% yield): mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 7.85 (d, J = 6.1 Hz, 1H), 7.04 (d, J = 6.1 Hz, 1H), 6.34 (s, 2H), 4.16 (s, 3H); ^{13}C NMR (DMSO-d_6), δ 166.4, 154.3, 148.7, 143.25, 102.4, 55.6.

3.1.23. 2-Amino-8-ethoxy-5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidine (5c)

The intermediate 3-amino-8-ethoxy-5-methylthio-1,2,4-triazolo[4,3-*c*]pyrimidine hydrobromide (**9c**) was isolated as pale yellow powder (90% yield): mp 160–163 °C; ^{1}H NMR (DMSO-d_6) δ 7.57 (s, 1H), 4.24 (q, J = 7.1 Hz, 2H), 2.68 (s, 3H), 1.40 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 147.2, 141.8, 140.9, 139.3, 121.6, 65.6, 14.2. The crude product was purified by column chromatography (SiO_2, acetone/hexanes, 60:40) to afford **5c** as light tan powder (55% yield): mp 190–191 °C; ^{1}H NMR (DMSO-d_6) δ 7.48 (s, 1H), 6.27 (s, 2H), 4.18 (q, J = 7.1 Hz, 2H), 4.07 (s, 3H), 1.35 (t, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6,) δ 166.0, 148.8, 143.9, 137.5, 124.8, 66.5, 55.4, 14.6.

3.1.24. 2-Amino-8-chloro-5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidine (5d)

The intermediate 3-amino-8-chloro-5-methylthio-1,2,4-triazolo[4,3-*c*]pyrimidine hydrobromide (**9d**) was isolated as a yellow powder (95% yield): mp >250 °C; ^{1}H NMR (DMSO-d_6), δ 8.22 (br s, 1H), 7.89 (s, 1H), 2.68 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 150.9, 147.9, 143.1, 138.4, 113.2, 14.2. The product (**5d**) was isolated as a tan solid (75% yield): mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 8.00 (s, 1H), 6.55 (s, 2H), 4.12 (s, 3H); ^{13}C NMR (DMSO-d_6), δ 166.4, 151.6, 147.7, 140.9, 108.5, 56.1. Anal. Calcd for $C_6H_6ClN_5O$: C, 36.11; H, 3.03; N, 35.09. Found: C, 36.11; H, 3.19; N, 34.82.

3.1.25. 2-Amino-8-fluoro-5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidine (5e)

The intermediate 3-amino-8-fluoro-5-methylthio-1,2,4-triazolo[4,3-*c*]pyrimidine hydrobromide (**9e**) was isolated as a yellow powder (82% yield): mp 168–170 °C; ^{1}H NMR (DMSO-d_6) δ 7.78 (d, J = 1.7 Hz, 1H), 7.10 (br s, 3H), 2.64 (s, 3H); The product (**5e**) was isolated as a tan solid (88% yield): mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 7.95 (d, J = 2.4 Hz, 1H), 6.53 (s, 2H), 4.10 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 166.4, 146.3, 145.4, 144.3, 127.5, 127.2, 56.0. Anal. Calcd for $C_6H_6FN_5O$: C, 39.35; H, 3.30; N, 38.24. Found: C, 39.33; H, 3.55; N, 37.89.

3.1.26. 2-Amino-5-methoxy-8-methyl-1,2,4-triazolo[1,5-*c*]pyrimidine (5f)

The intermediate 3-amino-8-methyl-5-methylthio-1,2,4-triazolo[4,3-*c*]pyrimidine hydrobromide (**9f**) was isolated as a yellow solid (95% yield): mp 234–236 °C; ^{1}H NMR (DMSO-d_6) δ 8.60 (s, 1H), 8.45 (br s, 3H), 3.53 (s, 3H), 3.10 (s, 3H); ^{13}C NMR (DMSO-d_6), δ 149.6, 147.9, 145.9, 113.8, 14.2, 12.0. The crude product was purified by column chromatography (SiO_2, CH_2Cl_2/EtOH, 95:5) to afford **5f** as a tan solid (26% yield): mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 7.63 (d, J = 1.1 Hz, 1H), 6.29 (s, 2H), 4.10 (s, 3H), 2.23 (d, J = 1.1 Hz, 3H); ^{13}C NMR (DMSO-d_6) δ 166.3, 154.5, 147.3, 140.7, 111.6, 55.3, 12.6. Anal. Calcd for $C_7H_9N_5O$: C, 46.92; H, 5.06; N, 39.08. Found: C, 46.70; H, 4.84; N, 39.09.

3.1.27. 2-Amino-7-fluoro-5-methoxy-1,2,4-triazolo[1,5-*c*]pyrimidine (5g)

The intermediate 3-amino-7-fluoro-5-methylthio-1,2,4-triazolo[4,3-*c*]pyrimidine hydrobromide (**9g**) was isolated as a tan powder (89% yield): mp 191–196 °C; ^{1}H NMR (DMSO-d_6) δ 8.00 (br s, 3H), 7.23 (d, J = 1.2 Hz, 1H), 2.77 (s, 3H). The crude product (**5g**) was isolated as a tan powder (84% yield): mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 6.75 (d, J = 1.1 Hz, 1H), 6.39 (s, 2H), 4.12 (s, 3H). Anal. Calcd for $C_6H_6FN_5O$: C, 39.35; H, 3.30; N, 38.24. Found: C, 39.55; H, 3.31; N, 38.18.

Compound **11b** was prepared according to the procedure for **11a** below:

3.1.28. 2-Propylthio-3-methoxymethoxy-trifluoromethylbenzene (11a)

A dry 2 L round bottom flask under N_2 was charged with anhydrous THF (500 mL), anhydrous TMEDA (67.8 mL, 449 mmol), anhydrous diisopropylamine (3.06 mL, 21.8 mmol), and 1-methoxymethoxy-3-trifluoromethylbenzene (90.0 g, 436 mmol). The resulting light yellow solution was cooled in a dry-ice/acetone bath (ca. −70 °C). To the cold solution was slowly added a solution of *n*-butyllithium (174 mL, 435 mmol, 2.5 M in hexane) such that the temperature remained between −65 °C and −70 °C. During the addition of *n*-butyllithium the solution turned a deep purple color. After all of the *n*-butyllithium had been added, the cold bath was removed and the solution allowed to warm to 0 °C, and this temperature was maintained for 2.5 h. The solution was again cooled to −70 °C and propyl disulfide (75.2 mL, 480 mmol) was slowly added. The resulting solution was warmed to room temperature, during which time a brown solid precipitates. After 18 h at room temperature, a portion of the volatiles (750 mL) was removed in vacuo. The remaining residue was diluted with Et_2O (700 mL) and washed with water (3 × 400 mL). The Et_2O solution was

dried ($MgSO_4$), filtered, and the solvent removed in vacuo to afford a crude gold oil. A GC analysis of the oil indicated a mixture of starting material, propyl disulfide and **11a**. The starting material and propyl disulfide were removed by distillation at reduced pressure (75 °C, 10 mm Hg) leaving the product as a tan oil (119 g, 97% yield) that was used without purification: ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 3H), 5.3 (s, 2H), 3.53 (s, 3H), 2.87 (t, J = 7.4 Hz, 2H), 1.55 (m, 2H), 0.96 (t, J = 7.1 Hz, 3H); GC–MS (EI, 70 eV) m/z 280 ($[M]^+$).

3.1.29. 2-Propylthio-3-trifluoromethylanisole (11b)

Distillation of the crude product after workup gave **11b** as a clear oil (71% yield): bp 92 °C (ca. 1 mm Hg); ^{1}H NMR ($CDCl_3$) δ 7.34–7.22 (m, 2H), 7.03 (d, J = 8.0 Hz, 1H), 2.80 (t, J = 7.5 Hz, 2H), 1.50 (s, J = 7.4 Hz, 2H), 0.93 (t, J = 7.5 Hz, 3H). Anal. Calcd for $C_{11}H_{13}F_3OS$: C, 52.79; H, 5.24; S, 12.81. Found: C, 52.65; H, 5.11; S, 12.93.

3.1.30. 2-Propylthio-3-trifluoromethylphenol (12)

To a solution of **11a** (119 g, 424 mmol) in methanol (400 mL) was added a concentrated solution of HCl (5.0 mL, 37 wt %) and the resulting solution was heated to 35 °C. After 3 d the solvent was removed in vacuo to give a dark oil. The oil was dissolved in CH_2Cl_2 (500 mL) and the solution washed with water (300 mL). The organics were dried ($MgSO_4$), filtered, and the solvent removed in vacuo to afford the crude product as a brown oil. The oil was distilled at reduced pressure (Kugelrohr, bp 70 °C, ca. 1.0 mm Hg) to afford **12** as a clear oil (84.5 g, 84% yield): ^{1}H NMR ($CDCl_3$) δ 7.48 (s, 1H), 7.37 (t, J = 8.0 Hz, 1H), 7.27 (dd, J = 1.1, 8.2 Hz, 1H), 7.21 (dd, J = 1.1, 8.0 Hz, 1H), 2.69 (t, J = 7.4 Hz, 2H), 1.63 (s, J = 7.4 Hz, 2H), 1.0 (t, J = 7.4 Hz, 3H).

Compounds **13b–f** were prepared according to the procedure for **13a** below:

3.1.31. 1-Ethoxy-6-trifluoromethylbenzene propyl sulfide (13a)

To a suspension of NaH (1.0 g, 25.4 mmol, 60% dispersion in mineral oil) in anhydrous DMF (20 mL), under an N_2 atmosphere, was slowly added **12** (5.0 g, 21 mmol). To the resulting solution was slowly added iodoethane (2.2 mL, 27 mmol). After a few minutes a solid began to precipitate and additional DMF (5 mL) was added to dissolve the precipitate. The resulting solution was stirred for 66 h. The mixture was diluted with H_2O (100 mL) and extracted with Et_2O (200 mL). The Et_2O solution was washed with H_2O (4 × 200 mL), dried ($MgSO_4$), filtered, and the solvent removed in vacuo to afford the crude product as a tan oil. The oil was distilled at reduced pressure (Kugelrohr, bp 90–100 °C, ca. 0.3 mm Hg) to afford **13a** as a colorless oil (5.1 g, 91% yield): ^{1}H NMR ($CDCl_3$) δ 7.34–7.26 (m, 2H), 7.04 (d, J = 6.6 Hz, 1H), 4.15 (q, J = 7.0 Hz, 2H), 2.87 (t, J = 7.5 Hz, 2H), 1.58–1.50 (m, 5H), 0.97 (t, J = 7.3 Hz, 3H); GC–MS (EI, 70 eV) m/z 264 ($[M]^+$).

3.1.32. 1-(2-Fluoroethoxy)-6-trifluoromethylbenzene propyl sulfide (13b)

The product was isolated as a colorless oil (95% yield): ^{1}H NMR ($CDCl_3$) δ 7.33–7.29 (m, 2H), 7.03 (dd, J = 3.8, 9.6 Hz, 1H), 4.80 (dt, J = 2.8, 47.3 Hz, 2H), 4.34–4.23 (m, 2H), 2.86 (t, J = 7.3 Hz, 2H), 1.50 (m, 2H), 0.93 (t, J = 7.3 Hz, 3H); GC–MS (EI) m/z 282 ($[M]^+$).

3.1.33. 1-(2,2,2-Trifluoroethoxy)-6-trifluoromethylbenzene propyl sulfide (13c)

The product was isolated as a colorless oil (65% yield): bp 73–76 °C (0.2 mm Hg); ^{1}H NMR ($CDCl_3$) δ 7.40–7.35 (m, 2H), 7.05 (d, J = 8.0 Hz, 1H), 4.43 (q, J = 8.1 Hz, 2H), 2.84 (t, J = 7.4 Hz, 2H), 1.51 (m, 2H), 0.93 (t, J = 7.2 Hz, 3H).

3.1.34. 1-[2-Fluoro-1-(fluoromethyl)ethoxy]-6-trifluoromethylbenzene propyl sulfide (13d)

The product was isolated as a colorless oil (49% yield): ^{1}H NMR ($CDCl_3$) δ 7.41 (dd, J = 2.0, 7.9 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.20 (dd, J = 1.9, 7.7 Hz, 1H), 4.82 (m, 2H), 4.76 (m, 1H), 4.67 (m, 2H), 2.87 (t, J = 7.4 Hz, 2H), 1.53 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H); GC–MS (EI) m/z 314 ($[M]^+$). Anal. Calcd for $C_{13}H_{15}F_5OS$: C, 49.68; H, 4.81. Found: C, 49.66; H, 4.79.

3.1.35. 1-(2,2-Difluoroethoxy)-6-trifluoromethylbenzene propyl sulfide (13e)

The product was isolated as a colorless oil (78% yield): bp 100 °C (0.5 mm Hg); ^{1}H NMR ($CDCl_3$) δ 7.38 (m, 2H), 7.05 (m, 1H), 6.20 (tt, J = 4.1, 54.9 Hz, 1H), 4.28 (td, J = 4.2, 12.9 Hz, 2H), 2.85 (t, J = 7.4 Hz, 2H), 1.54 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H); GC–MS (EI) m/z 300 ($[M]^+$). Anal. Calcd for $C_{12}H_{13}F_5OS$: C, 48.00; H, 4.36; S, 10.68. Found: C, 47.92; H, 4.46; S, 10.76.

3.1.36. 1-(3-Fluoropropoxy)-6-trifluoromethylbenzene propyl sulfide (13f)

The product was isolated as a colorless oil (97% yield): ^{1}H NMR ($CDCl_3$) δ 7.39–7.30 (m, 2H), 7.08 (dd, J = 1.7, 6.0 Hz, 1H), 4.73 (dt, J = 5.6, 47.0 Hz, 2H), 4.21 (t, J = 6.2 Hz, 2H), 2.82 (t, J = 7.3 Hz, 2H), 2.26 (dp, J = 5.9, 26.1 Hz, 2H), 1.54 (m, 2H), 0.96 (t, J = 7.4 Hz, 3H); GC–MS (EI) m/z 296 ($[M]^+$).

Compounds **14b–h** were prepared according to the procedure for **14a** below:

3.1.37. 2-Ethoxy-6-trifluoromethylbenzenesulfonyl chloride (14a)

Compound **13a** (5.1 g, 19.3 mmol) was dissolved in a solution of acetic acid (20 mL) and H_2O (1.5 mL), and the resulting solution was heated to 45 °C. To the warm solution was slowly added Cl_2 (10.2 g, 144 mmol). During the addition of Cl_2 the temperature of the solution rose to 55 °C. After all of the Cl_2 had been added, the temperature of the solution was maintained at 55 °C (ca. 2 h). The solution was then poured into ice water and the cold mixture extracted with Et_2O (150 mL). The Et_2O layer was dried ($MgSO_4$), filtered, and the solvent removed in vacuo to afford a yellow oil. The oil was purified by column chromatography (SiO_2, 0→20% EtOAc/hexane) to afford **14a** as light yellow crystals (4.5 g, 81% yield): mp 52–54 °C; ^{1}H NMR ($CDCl_3$) δ 7.70 (t, J = 8.0 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 4.34–4.27 (q, J = 7.0 Hz, 2H), 1.55 (t, J = 7.0 Hz, 3H).

3.1.38. 2-Methoxy-6-fluorobenzenesulfonyl chloride (14b)

The product was isolated as a tan oil (76% yield): ^{1}H NMR ($CDCl_3$) δ 7.62–7.55 (m, 1H), 6.88–6.77 (m, 2H), 4.02 (s, 3H).

3.1.39. 2-Methoxy-6-trifluoromethylbenzenesulfonyl chloride (14c)

The product was isolated as a white solid (54% yield): mp 86–88 °C; ^{1}H NMR ($CDCl_3$) δ 7.82 (m, 1H), 7.53 (d, J = 7.3 Hz, 1H), 7.46 (d, J = 8.6 Hz, 1H), 4.14 (s, 3H).

3.1.40. 1-(2-Fluoroethoxy)-6-trifluoromethylbenzene sulfonyl chloride (14d)

The product was isolated as a white solid (90% yield): mp 69–72 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (t, J = 8.7 Hz, 1H), 7.52 (d, J = 7.7 Hz, 1H), 7.42 (d, J = 8.6 Hz, 1H), 4.86 (dt, J = 4.04, 7.2 Hz, 2H), 4.48 (dt, J = 4.0, 27.0 Hz, 2H).

3.1.41. 1-(2,2,2-Trifluoroethoxy)-6-trifluoromethylbenzene sulfonyl chloride (14e)

The product was isolated as a white solid (94% yield): mp 59–63 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (t, J = 7.7, 8.7 Hz, 1H), 7.64 (d, J = 7.3 Hz, 1H), 7.39 (d, J = 8.3 Hz, 1H), 4.58 (q, J = 7.8 Hz, 2H).

3.1.42. 1-[2-Fluoro-1-(fluoromethyl)ethoxy]-6-trifluoromethylbenzene sulfonyl chloride (14f)

The product was isolated as a light yellow solid (100% yield): mp 65–70 °C (d); ^{1}H NMR ($CDCl_3$) δ 7.77 (td, J = 0.8, 8.5 Hz, 1H), 7.57 (t, J = 8.0 Hz, 2H), 5.07 (m, J = 5.2 Hz, 1H), 4.88 (m, 2H), 4.72 (m, 2H); MS-DIP (CI) m/z 338 ([M]$^+$). Anal. Calcd for $C_{10}H_8ClF_5O_3S$: C, 35.46; H, 2.38; S, 9.47. Found: C, 35.24; H, 2.53; S, 9.41.

3.1.43. 1-(2,2-Difluoroethoxy)-6-trifluoromethylbenzene sulfonyl chloride (14g)

The product was isolated as a white solid (100% yield): mp 66–68 °C; ^{1}H NMR ($CDCl_3$) δ 7.81 (td, J = 0.5, 8.5 Hz, 1H), 7.62 (dd, J = 0.5, 7.9 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 6.26 (tt, J = 4.1, 54.9 Hz, 1H), 4.45 (td, J = 4.1, 12.3 Hz, 2H). Anal. Calcd for $C_9H_6ClF_5O_3S$: C, 48.00; H, 4.36; S, 10.68. Found: C, 47.92; H, 4.46; S, 10.76.

3.1.44. 1-(3-Fluoropropoxy)-6-trifluoromethylbenzene sulfonyl chloride (14h)

The product was isolated as a light yellow oil (81%): ^{1}H NMR ($CDCl_3$) δ 7.77 (t, J = 8.0, 8.5 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 4.77 (dt, J = 5.5, 47.0 Hz, 2H), 4.38 (t, J = 6.0 Hz, 2H), 2.32 (dp, J = 5.8, 26.1 Hz, 2H); GC–MS (EI) m/z 320 ([M]$^+$).

3.1.45. 2,6-Dimethoxybenzenesulfonyl chloride (16)

A 1 L flask fitted with an addition funnel, thermometer and mechanical stirrer was charged with 1,3-dimethoxybenzene (**15**, 14.2 mL, 108 mmol), anhydrous tetramethylethylenediamine (TMEDA, 18 mL, 119 mmol) and petroleum ether (225 mL). The resulting solution was cooled in an ice/water bath. A solution of *n*-butyllithium (47.5 mL, 119 mmol, 2.5 M in hexanes) was slowly added from the addition funnel such that the temperature did not rise above 5 °C. The reaction mixture was stirred at 0 °C for 20 min and then cooled in a dry-ice/acetone bath (−70 °C). To the resulting mixture was added a cold (−65 °C) solution of SO_2 (70 g, 1.08 mol) in dry Et_2O (100 mL) in portions such that the temperature did not rise above −60 °C. The light yellow mixture was slowly warmed to 10 °C, and the solids were filtered and washed with dry Et_2O. The resulting solids were suspended in hexane (400 mL) and cooled to 0 °C. To the cold suspension was added a solution of sulfuryl chloride (14.5 g, 108 mmol) in hexane (200 mL) in portions such that the temperature did not rise above 3 °C. The resulting mixture was allowed to stir at 0 °C 1 h, and then filtered, and the resulting filter cake washed with cold hexane. The solids were mixed with Et_2O (600 mL) and the Et_2O mixture washed with H_2O (600 mL). The layers were separated, the aqueous phase extracted with additional Et_2O, and the organics were combined, dried ($MgSO_4$), filtered and the solvent removed in vacuo to afford the crude product as a light yellow solid (19.42 g, 76% yield): mp 89–91 °C; ^{1}H NMR ($CDCl_3$) δ 7.51 (t, J = 8.5 Hz, 1H) 6.64 (d, J = 8.5 Hz, 2H) 3.92 (s, 6H); GC–MS (EI, 70 eV) m/z 236 ([M]$^+$).

3.2. Biology data

3.2.1. General procedure for post-emergence tests (Tables 1 and 2)

3.2.1.1. Plant material. For post-emergence evaluations, seeds of desired test species were planted in Grace-Sierra Metromix 306 (pH 6.0–6.8, O.M. 30%) in square plastic pots with a surface area of 64 cm^2. Chemical or physical seed treatments were used on some species to promote germination. Plants were grown for 7 to 21 days in greenhouses typically maintained on a 23–29 °C day/22–28 °C night thermoperiod and a 15 h photoperiod. Chemicals were applied when plants were 2–12 cm in height and in the 1–4 true leaf stage of growth. Exact height was species dependent. Following chemical application, plants were maintained in a greenhouse on a 23 °C day/22 °C night thermoperiod and a 15 h photoperiod.

Supplemental lighting for post-emergence tests was provided with 1000 W metal halide overhead lamps which gave average illumination of 500 uEM^{-2} s^{-1} PAR at the plant canopy. Plants treated post-emergence were watered by sub-irrigation to prevent washing the chemical off the foliage. Plants were watered with fertilizer solution 5–7 times per week to maintain good plant growth.

3.2.1.2. Formulation and application of chemicals. Stock solutions of sulfonamide compounds were formed by dissolving samples in a solution of acetone/DMSO (97:3 v/v). Post-emergence spray solutions were formulated by injecting aliquots of stock solution into spray solution comprised of acetone/water/isopropanol/DMSO/Agridex/Triton X-155 (48.5:39:10:1.5:1.0:0.02 v/v). Applications were applied by spraying the foliage of test plants with a DeVilbiss atomizer driven by compressed air at a pressure of 14–28 kPa. Approximately 1.5 mL of spray solution was applied to the plants in each pot. The volume of spray and adjuvant provided thorough spray coverage.

3.2.1.3. Assessment. Unless otherwise noted, the in vivo greenhouse screening data were a tabulation of post-emergence foliar applied results and expressed as a 'percent in growth reduction' (GR) for treated plants, where 0 is no injury and 100 is complete kill, as visually compared to untreated plants. Assessments of phytotoxic effects were made two weeks after chemicals were applied in post-emergence tests. The broadleaf weed activity (BW) is given as an average of 80% reduction in growth at a given concentration, expressed in parts per million (ppm), over five to eight broadleaf weeds chosen from the following: *Xanthium strumarium*, *Datura stramonium*, *Chenopodium album*, *Helianthus* spp., *Ipomoea* spp., *Amaranthus retroflexus*, *Abutilon theophrasti*, *Veronica heteraefolia*, *Ipomoea hederacea*, *Stellaria media* and *Polygonum convolvulus*. The grass weed activity (GW) is averaged over five weeds chosen from *Alopecurus* spp., *E. crus-galli*, *Setaria fabari*, *Sorghum halapense*, *Digitaria sanguinalis* and *Avena fatua* and expressed in a manner similar to broadleaf weeds. The injury for a specified crop is expressed as a 20% reduction in growth compared to the untreated crop.

3.2.2. General procedure for water injection assay (Table 3)

In the field, puddled soil (saturated soil that has had its structure destroyed by constant mixing) provides a low-leaching, level, and uniform soil layer which is essential to the root growth of the young (usually 2–3 leaf) rice seedling transplants. In the greenhouse, puddled soil was prepared to grow the different transplanted rice weeds to simulate a flooded rice paddy environment. A 32 oz (960 mL, 10.5 cm diameter) disposable cup containing 650 mL of mud per One pot was used to grow transplanted rice (*O. sativa*) and *E. crus-galli*. Other key rice weeds, *Monochoria vaginalis*, *Cyperus difformis*, and *Scirpus juncoides* were all grown in multiple 16 oz disposable cups with a volume of 250 mL of mud per cup. All cups used in the transplanted paddy rice screen are non-perforated to be able to maintain a constant depth of water, preferably 3 cm deep. The rice herbicide program uses a variety of non-sterilized mineral soils for its tests.

All weed seeds are either collected by field scientists or purchased. Prior to filling the 16 and 32 oz cups with mud, a measured amount of slow release fertilizer was added to the bottom of each cup. The slow release fertilizer was the only fertilizer that the plants received during the course of an experiment, since the addition of liquid fertilizer to the paddy flood will promote the growth of algae, making test evaluation very difficult. The amount of mud added to the cups allows for a 3 cm paddy flood depth to be

maintained above the mud surface during the course of greenhouse testing.

Rice seeds (*O. sativa*, japonica) were sown 3–8 seeds per cell in a 120 cell plastic plug tray (25 cm × 50 cm × 4.5 cm, cell dimension = 2.5 cm × 2.5 cm) that had been filled with Metro Mix 360, a soil-less planting medium consisting of peat moss, vermiculite and fir bark with the top 1/4 in. of Metro Mix brushed off and the tray sub-watered until the mix was completely saturated. After the seeds were sown on the saturated Metro Mix, they were covered with approximately 0.5 cm Metro Mix. The covered seed was lightly top watered and the trays were incubated at high temperature and humidity (30 °C, 95% RH) for 48 h in the dark, rotating the trays at 24 h to maintain uniformity. During this time the rice seed should germinate and have approximately 1.5–2 cm of etiolated growth visible above the Metro Mix prior to removal from the germination chamber and placed in the rice greenhouse. The flats remain in the greenhouse an additional 88–96 h before being transplanted into the deli cups. The day after they are moved into the greenhouse, the flats were fertilized twice with a solution of 1/4 tsp (2.5 g) ammonium sulfate + 1/4 tsp (1.8 g) iron chelate in 1 gallon (3.7 L) water. If not fertilized with this liquid fertilizer, rice seedlings will grow poorly and be a nice shade of yellow instead of a healthy green color. When the rice was at the 2–3 leaf stage, typically at 6 d after seeding in the Metro Mix in the cell flat, it was ready for transplanting. Cells of the plug trays were removed intact and the remaining Metro Mix was washed off in water and the seedlings grouped into hills or clusters of seedlings consisting of 3 seedlings per hill or cluster, with a 32 oz deli cup planted with 2 hills. Each hill was pushed into the soil, submerging the roots and seed hulls to a depth of about 2 cm from seed hull to soil surface. After all cups have been transplanted, the cups were flooded to a depth of 3 cm. The transplants were allowed to recover and develop an additional 0.5–1 leaf prior to treatment. This usually requires 5 d. The entire process from seed soaking to treatment typically requires 15 d.

Application rates were calculated based on the surface area of the treated pot. Application of each treatment was made by injection of the treatment solution with an Eppendorf positive displacement pipette. A serial dilution was used to apply the different rates tested for each active ingredient, achieved by changing the treatment volume delivered to each pot, where the high rate receives 4 mL of the stock solution per pot, the second rate 2 mL per pot, etc. Following treatment, the flood depth in the cups was held constant at 3 cm. It was very important not to overfill the cups for 2 days after treatment, so as not to wash out the treatment. The 3 cm depth was maintained by addition of water at least twice a day. De-ionized water was used in the rice greenhouse to prevent buildup of salts and dissolved solids. This 3 cm depth was representative of commercial applications of actual herbicides in flooded transplanted paddy rice in Japan and Korea.

All tests were incubated in the greenhouse where conditions were maintained on a 16 h day length with a daytime/nighttime demand temperature of 29 °C (88 °F). Tests were typically evaluated 3 weeks after application to allow sufficient time for weed and rice to express maximum symptoms. Visual evaluations of test results were made based on a scale of 0–100%, where 0% is no injury or growth reduction and 100% is complete death.

3.2.3. General procedure for ALS enzyme extraction and assay

The following procedures were adapted from Muhitch et al.[25] and were performed at 0–4 °C. The etiolated shoots of 5–6 d old Hector Spring barley (100 g) were homogenized in a Waring Blender for 2 min with 2.5 volumes of extraction buffer containing 20 mM HEPES (pH 7.2), 1 mM EDTA, and 5 mM DTT. The homogenate was filtered through 2 layers of Miracloth and 4 layers of cheesecloth and centrifuged at 25,000*g* for 20 min. The supernatant was filtered through 2 layers of Miracloth and brought to 33% saturation with $(NH_4)_2SO_4$ while stirring for 40 min. After centrifugation at 25,000*g* for 20 min, the supernatant was discarded and the pellet was re-suspended in 3 mL of extraction buffer. The re-suspended pellet could then be frozen in liquid N_2 and stored at −80 °C for up to 1 month before use.

Enzyme activity was assayed colorimetrically by measuring the amount of acetoin formed from acetolactate using the method of Westerfeld.[26] Standard reaction mixtures contained 20 mM HEPES (pH 7.2), 5 mM $MgCl_2$, 0.25 mM TPP, 3.3 μM FAD, 22.5 mM sodium pyruvate and various concentrations of the inhibitors in a final DMSO concentration of 0.7%. The reaction was initiated with the enzyme and the mixture was incubated at 37 °C for 30 min. The reaction was stopped with the addition of H_2SO_4 to produce a final concentration of 0.5% H_2SO_4 and heated at 60 °C for 20 min to decarboxylate the acetolactate. The acetoin concentration produced was determined by adding creatine and 1-naphthol to the reaction mixture in final concentrations of 2.0 mg/mL and 20 mg/mL, respectively. The mixture was made alkaline with the addition of NaOH to a final concentration of 0.5 N and incubated at 37 °C for 40 min. The absorbency was measured at 530 nm to determine the amount of acetoin produced. The reaction was determined to be linear for at least 75 min. The in vitro inhibition of acetolactate synthase is indicated by 50% inhibition of the enzyme (I_{50}) in nanoMolar units (nM).

References and notes

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