Regular Article

Design, Synthesis and Biological Evaluation of Novel Pyrazole Sulfonamide Derivatives as Potential AHAS Inhibitors

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Acetohydroxy acid synthase (AHAS; EC 2.2.1.6, also referred to as acetolactate synthase, ALS) has been considered as an attractive target for the design of herbicides. In this work, an optimized pyrazole sulfonamide base scaffold was designed and introduced to derive novel potential AHAS inhibitors by introducing a pyrazole ring in flucarbazone. The results of *in vivo* herbicidal activity evaluation indicates compound 3b has the most potent activity with rape root length inhibition values of 81% at 100 mg/L, and exhibited the best inhibitory ability against *Arabidopsis thaliana* AHAS. With molecular docking, compound 3b insert into *Arabidopsis thaliana* AHAS stably by an H-bond with Arg377 and cation–π interactions with Arg377, Trp574, Tyr579. This study suggests that compound 3b may serve as a potential AHAS inhibitor which can be used as a novel herbicides and provides valuable clues for the further design and optimization of AHAS inhibitors.

Key words pyrazole sulfonamide; acetohydroxy acid synthase; herbicidal activity; molecule docking

The branched-chain amino acids (BCAAs) biosynthetic pathway exists only in plants and micro-organisms, not in the bodies of mammals, and acetohydroxy acid synthase (AHAS; EC 2.2.1.6, also referred to as acetolactate synthase, ALS) plays an important role in the BCAAs biosynthetic pathway.[1,2] For those reasons, made AHAS an ideal targets for the design of "green herbicides" since the mid-1980s.[3–5] There are four main categories of listed AHAS inhibitors, including: sulfonylureas, imidazolinones, pyrimidinylthio (or oxo) benzoates and triazolopyrimidine sulfonanilides. However, the frequent use of these herbicides has caused mutations in weed AHAS.[6–9] Therefore, the discovery of new drug groups with AHAS inhibitory activity is particularly urgent.

For this reason, we chose AHAS as target, and introduced pyrazole ring instead of the triazole ring in the flucarbazone molecule (Fig. 1). The attractiveness of pyrazole and its derivatives is their versatility that allows for synthesis of a series of analogues with different moieties in them, thus affecting the electronics and by extension the properties of the resultant compounds.[10,11] It is widely used as a large number of compounds for various applications, such agrochemicals[12–14] and medicine,[15–18] due to their broad range of biological activities. Therefore, it is expected that the introduction of the pyrazole ring can yield compounds with excellent herbicidal activity.

Herein, we describe the design and synthesis of a series of pyrazole sulfonamide derivatives by introducing a pyrazole ring into the flucarbazone molecule. The *in vivo* herbicidal activities of these compounds were evaluated, and subsequently molecular docking was performed for exploring the binding mode between small molecules and the *Arabidopsis thaliana* AHAS (*At*AHAS).

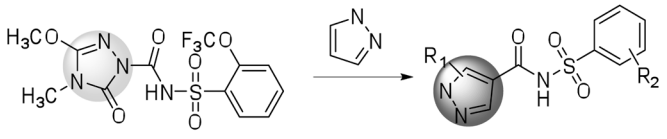
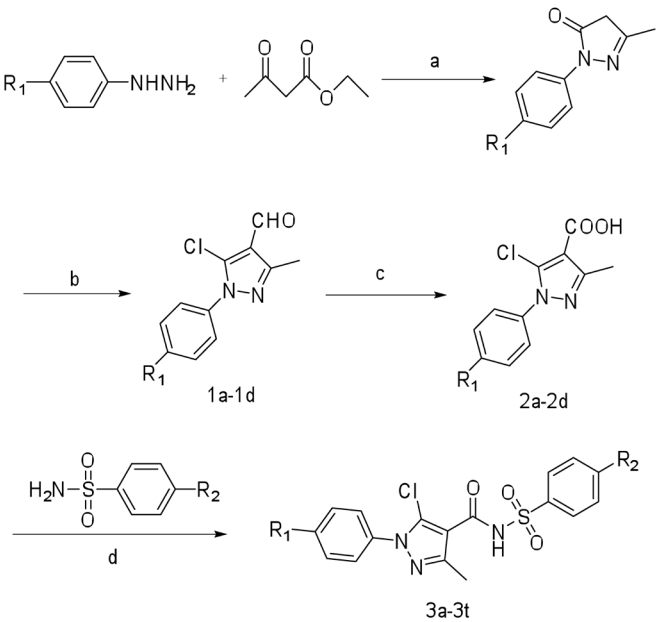


Fig. 1. Design of Target Compounds

Results and Discussion

Chemistry A series of novel pyrazole sulfonamide derivatives was synthesised and the general pathway is outlined in Chart 1. After cyclization of substituted phenylhydrazine



Reagents and conditions: (a) H_2O, ethanol, 60°C; (b) DMF, $POCl_3$, 90°C, 5 h; (c) $KMnO_4$, 70–80°C; (d) EDCI, HOBt, DMF, r.t.

Chart 1. General Synthesis of Compounds **4a**–**4t** and **5a**–**5p**

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Table 1. *In Vivo* Herbicidal Activity of the Compounds **3a**–**3t**

Compound	R_1	R_2	Rape root length inhibition (%)	CDOCKER_ Interaction energy (kcal mol^{-1})[a)]
			100 mg/L	
3a	H	H	0	−23.2624
3b	H	Cl	81	−31.9225
3c	H	CH_3	12	−24.5746
3d	H	OCH_3	43	−26.8199
3e	H	OCF_3	68	−27.5274
3f	F	H	0	−24.3675
3g	F	Cl	13	−26.0059
3h	F	CH_3	25	−26.6266
3i	F	OCH_3	36	−24.7947
3j	F	OCF_3	33	−26.9420
3k	Cl	H	26	−24.0369
3l	Cl	Cl	21	−24.6165
3m	Cl	CH_3	16	−26.7611
3n	Cl	OCH_3	72	−31.0646
3o	Cl	OCF_3	54	−28.9238
3p	CH_3	H	36	−24.8901
3q	CH_3	Cl	10	−24.5399
3r	CH_3	CH_3	0	−23.5403
3s	CH_3	OCH_3	19	−23.4165
3t	CH_3	OCF_3	46	−24.2396
Monosulfuron			87	

a) Internal ligand strain energy and receptor–ligand interaction energy obtained from the docking study of all synthesized compounds by the CDOCKER protocol (Discovery Studio 3.1, Accelrys, Inc., San Diego, CA, U.S.A.).

and ethylacetoacetate in ethanol, **1a**–**1d** was obtained with Vilsmeier–Haack reagent (*N*,*N*-dimethylformamide (DMF)–$POCl_3$). Then **1a**–**1d** were oxidized by $KMnO_4$ solution to obtain **2a**–**2d**. The synthesis of the target compounds was carried out through our previously reported method.[19] All the synthetic compounds were analysed by element and spectroscopic methods, which showed that all compounds are in full accordance with the structures depicted in Table 1.

Biological Activity The *in vivo* herbicidal activity of the compounds **3a**–**3t** was measured by using the rape root inhibition method and the measurement results are summarized in Table 1. The sulfonylurea herbicide monosulfuron was used as the control.

These results show that most of the compounds exhibited different levels of *in vivo* herbicidal activities at a concentration of 100 mg L^{-1}. The most potent of the compounds **3b**, **3e** and **3n** showed 81, 68 and 72% inhibitory activities respectively, slightly lower than 87% inhibitory activity of monosulfuron. Furthermore, compounds **3d**, **3o** and **3t** (43, 54, 46%) showed about 50% inhibition activities, and the remaining compounds have 0–40% inhibitory activities. However, it is regrettable that some of the compounds showed no inhibitory activity slightest, such as compounds **3a**, **3f** and **3r**.

According to the *in vivo* herbicidal activity as Table 1, three compounds tested against *At*AHAS activity *in vitro* which *in vivo* herbicidal activity values of >60% and the measurement results are summarized in Table 2. All of the compounds showed excellent inhibitory activity against *At*AHAS as Table 2. Compound **3e** showed the best inhibitory ability against *At*AHAS with values of 81% at 100 mg/L, compounds **3b**, **3n** show greater than 60% inhibitory abilities against *At*AHAS.

Table 2. *In Vitro At*AHAS Inhibition of the Compounds

Compound	*At*AHAS inhibition (%)
	100 mg/L
3b	65
3e	81
3n	73
Monosulfuron	93

However, the inhibitory activity of these compounds is lower than that of monosulfuron.

Molecular Docking In order to verify this assumption, molecular docking was performed by target compounds into the binding site of *At*AHAS (PDB entry: 5k6t).[20] To our delight, it was clearly seen that compounds **3b** and **3n** showed obviously lower interaction energy than positive drugs flucarbazone, demonstrating that these compounds are likely to exhibit more potent inhibitory activity against *At*AHAS (Table 1). These preliminary Computer Aided Drug Design (CADD) results suggested these design pyrazole derivatives containing sulfonamide moiety possibly served as potent herbicides by inhibiting AHAS.

We explored their binding model generated by molecular docking based on the *At*AHAS (PDB entry: 5k6t) and preprocessed by the DS 3.1 (Discovery Studio 3.1, Accelrys, Inc.). As showed in Fig. 2(A), the compound **3b** skeleton was deeply embedded into the binding pocket, suggesting the pose of **3b** into the *At*AHAS-binding site which revealed that it has suitable shape complementarity with the binding pocket, which means that the reasonableness of our design molecules. As showed in Fig. 2(B), cationic sidechain Arg377 makes cation–π interaction with **3b**. The results of docking simulation suggested there were the same binding mode that the presence of flucarbazone molecular in a 5k6t crystal protein, with electron-withdrawing group ester and sulfonyl groups on its benzene ring, still produces a cation–π interaction with Arg377 (Figure S uploaded as an attachment).[20] In addition, Trp574 and Tyr579 are combined with electron-rich π system of benzene and pyrazole ring in compound **3b**, another stable H-bond was also detected in the binding model between O in the sulfonamide and Arg377 (angle=116.9°, distance=1.8 Å), which provides valuable information for the further design of AHAS inhibitors.

Conclusion

A series of novel pyrazole sulfonamide derivatives were synthesised and evaluated for their *in vivo* herbicidal activities and *in vitro At*AHAS inhibition activities. Compound **3b** showed the most potent *in vivo* herbicidal activity with values of 81% at 100 mg/L, and exhibited the best inhibitory ability against *At*AHAS. Docking simulation showed compound **3b** skeleton was deeply embedded into the binding pocket. Above all, the results obtained from this study suggest that compound **3b** may serve as a potential AHAS inhibitors which can be used as a novel herbicides and provide valuable information for the design AHAS inhibitors.

Experimental

Materials and Measurements All chemicals (reagent grade) used were purchased from Sigma-Aldrich (St. Louis,

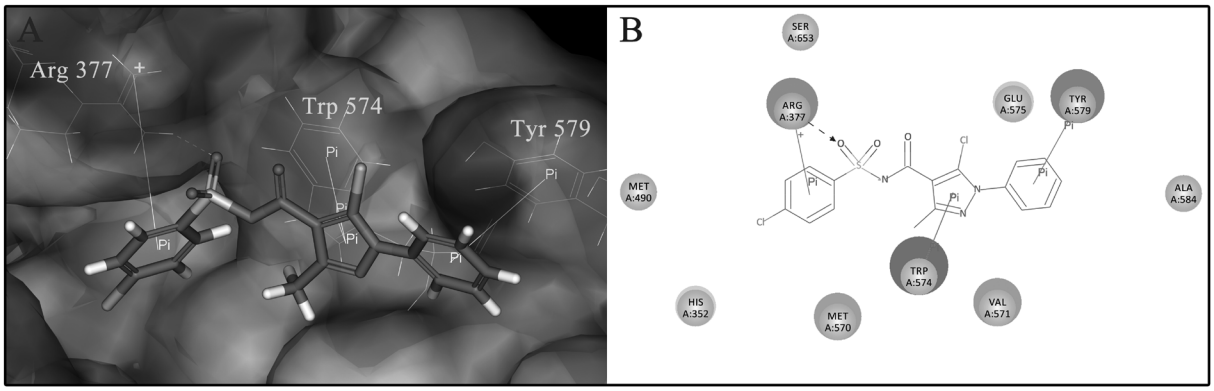

Fig. 2. Binding Model of **3b** in the Active Site of *At*AHAS (PDB Entry: 5k6t)

The H-bond is displayed as dashed line and π interaction is displayed as solid line.

MO, U.S.A.) and Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). All the ^{1}H-NMR spectra were measured on an Agilent DD2 600 Hz spectrometer (Agilent Technologies Corporation, Santa Clara, CA, U.S.A.). Chemical shifts were reported in ppm (δ). Electrospray ionization (ESI)-MS spectra were recorded on a Mariner System 5304 mass spectrometer (Applied Biosystems, Foster City, CA, U.S.A.). Elemental analyses were performed on a CHN-O-Rapid instrument (Leco, Tres Cantos, Madrid, Spain) and were within 0.4% of the theoretical values. Melting points were measured without correction.

General Procedure for Synthesis of 5-Chloro-1-aryl-3-methyl-1*H*-pyrazole-4-carboxylic Acids 2a–2d[21] *para*-Substituted phenyl hydrazine (25 mmol) was dissolved in anhydrous ethanol, ethyl acetoacetate (25 mmol) was slowly added and stirred at 70–80°C for 5 h, then the anhydrous ethanol was removed under reduced pressure to form a solid, which was dissolved in DMF (20 mL) and phosphorus oxychloride (16 mL) of cold mixed solution and stirred at 90°C for 1h. The resulting mixture was poured into ice-cold water, the resulting solid was separated by filtration to give the light yellow solid. Then above product was oxidized by 5.0 mol/L $KMnO_4$ solution, stirred at 70–80°C. After cooling to room temperature the pH of the reaction mixture was adjusted to pH 7–8 by the dropwise addition of 3.0 mol/L KOH solution, and the solution was filtered, 6.0 mol/L HCl solution was added to the solution and solid **2a**–**2d** eventually separated out. The crude product obtained was recrystallized from anhydrous ethanol to afford the pure product.

General Procedure for Synthesis of 5-Chloro-3-methyl-1-phenyl-*N*-(phenylsulfonyl)-1*H*-pyrazole-4-carboxamide 3a–3t To a stirred solution of the intermediates compound **2a**–**2d** (1 mmol) with triethylamine (2 mmol) into DMF (12 mL), then a mixture of 1-ethyl-(3-(3-dimethylamino)-propyl)-carbodiimide hydrochloride (EDCI) (1 mmol) and *N*-hydroxybenzotriazole (HOBt) (1 mmol) was placed in the reaction system, stirred at room temperature for 30 min, the mixture of substituted benzene sulfonamide (1 mmol) and DMF (5 mL) was added in the reaction system, the reaction mixture was monitored by TLC. After completion of the reaction, the product was extracted from chloroform with water, 0.2 mol/L hydrochloric acid, water, 2.0 mol/L sodium hydroxide, saturated sodium chloride successively, and then dried, concentrated, and purified by preparative thin layer chromatography followed by recrystallization from ethanol.

5-Chloro-3-methyl-1-phenyl-*N*-(phenylsulfonyl)-1*H*-pyrazole-4-carboxamide (**3a**)

Light yellow solid, yield 74%; mp 156–158°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.65 (s, 1H), 8.17 (d, J=8.0 Hz, 2H), 7.67 (t, J=7.4 Hz, 1H), 7.58 (t, J=7.7 Hz, 2H), 7.44 (dd, J=8.6, 4.7 Hz, 3H), 7.21 (t, J=8.3 Hz, 2H), 2.47 (s, 3H). ^{13}C-NMR (151 MHz, dimethyl sulfoxide (DMSO)) δ: 163.17, 150.21, 140.81, 138.04, 133.63, 131.93, 130.07, 129.02, 128.43, 128.31, 127.34, 116.83, 13.72. MS (ESI): 376.8 ($C_{17}H_{14}ClN_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{17}H_{14}ClN_3O_3S$: C, 54.33; H, 3.75; N, 11.18. Found: C, 54.36; H, 3.79; N, 11.25.

5-Chloro-*N*-((4-chlorophenyl)sulfonyl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide (**3b**)

Light yellow solid, yield 73%; mp 152–154°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.65 (s, 1H), 8.10 (d, J=8.6 Hz, 2H), 7.55 (d, J=8.6 Hz, 2H), 7.44 (dd, J=8.9, 4.7 Hz, 3H), 7.21 (t, J=8.4 Hz, 2H), 2.48 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.20, 150.20, 139.08, 138.82, 138.02, 133.63, 130.04, 129.79, 129.57, 128.40, 128.34, 116.80, 13.73. MS (ESI): 411.2 ($C_{17}H_{13}Cl_2N_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{17}H_{13}Cl_2N_3O_3S$: C, 49.77; H, 3.19; N, 10.24. Found: C, 49.79; H, 3.24; N, 10.31.

5-Chloro-3-methyl-1-phenyl-*N*-tosyl-1*H*-pyrazole-4-carboxamide (**3c**)

Light yellow solid, yield 71%; mp 131–134°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.67 (s, 1H), 8.03 (d, J=8.3 Hz, 2H), 7.44 (dd, J=8.9, 4.7 Hz, 3H), 7.37 (d, J=8.1 Hz, 2H), 7.20 (t, J=8.5 Hz, 2H), 2.47 (s, 3H), 2.45 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.17, 150.04, 137.99, 137.81, 137.64, 133.65, 130.02, 129.33, 128.37, 128.36, 128.11, 116.80, 21.56, 13.70. MS (ESI): 390.8 ($C_{18}H_{16}ClN_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{16}ClN_3O_3S$: C, 55.46; H, 4.14; N, 10.78. Found: C, 55.50; H, 4.19; N, 10.72.

5-Chloro-*N*-((4-methoxyphenyl)sulfonyl)-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxamide (**3d**)

Light yellow solid, yield 84%; mp 113–114°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.71 (s, 1H), 8.23 (d, J=8.9 Hz, 2H), 7.57–7.47 (m, 3H), 7.45 (d, J=6.8 Hz, 2H), 7.39 (d, J=8.7 Hz, 2H), 2.49 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.84, 163.17, 150.07, 133.21, 129.55, 129.38, 128.39, 126.13, 116.87, 116.72, 114.62, 56.81, 13.70. MS (ESI): 406.8 ($C_{18}H_{16}ClN_3O_4S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{16}ClN_3O_4S$: C, 53.27; H, 3.97; N, 10.35. Found: C, 53.31; H, 4.02; N, 10.28.

5-Chloro-3-methyl-1-phenyl-*N*-((4-(trifluoromethoxy)phenyl)-sulfonyl)-1*H*-pyrazole-4-carboxamide (**3e**)

Light yellow solid, yield 79%; mp 117–119°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.71 (s, 1H), 8.23 (d, J=8.6 Hz, 2H), 7.54–7.48 (m, 3H), 7.45 (d, J=7.3 Hz, 2H), 7.39 (d, J=8.4 Hz, 2H), 2.49 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.17, 153.83, 150.04, 133.13, 129.76, 129.55, 128.39, 129.38, 126.17, 116.87, 116.72, 114.65, 13.71. MS (ESI): 460.8 ($C_{18}H_{13}ClF_3N_3O_4S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{13}ClF_3N_3O_4S$: C, 47.02; H, 2.85; N, 9.14. Found: C, 47.11; H, 2.88; N, 9.21.

5-Chloro-1-(4-fluorophenyl)-3-methyl-*N*-(phenylsulfonyl)-1*H*-pyrazole-4-carboxamide (**3f**)

Light yellow solid, yield 80%; mp 158–160°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.65 (s, 1H), 8.16 (d, J=8.6 Hz, 2H), 7.67 (t, J=7.5 Hz, 1H), 7.58 (t, J=7.8 Hz, 2H), 7.44 (dd, J=8.9, 4.7 Hz, 2H), 7.21 (t, J=8.5 Hz, 2H), 2.47 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.17, 160.47, 150.22, 139.08, 138.82, 133.63, 132.81, 129.79, 129.57, 116.84, 116.12, 115.63, 13.70. MS (ESI): 394.8 ($C_{17}H_{13}ClFN_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{17}H_{13}ClFN_3O_3S$: C, 51.85; H, 3.33; N, 10.67. Found: C, 51.90; H, 3.38; N, 10.72.

5-Chloro-*N*-((4-chlorophenyl)sulfonyl)-1-(4-fluorophenyl)-3-methyl-1*H*-pyrazole-4-carboxamide (**3g**)

Light yellow solid, yield 81%; mp 157–159°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.67 (s, 1H), 8.10 (d, J=8.6 Hz, 2H), 7.55 (d, J=8.6 Hz, 2H), 7.44 (dd, J=8.9, 4.7 Hz, 2H), 7.21 (t, J=8.4 Hz, 2H), 2.47 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.17, 160.45, 150.21, 138.94, 137.52, 133.63, 132.81, 129.17, 128.75, 116.80, 116.18, 115.67, 13.71. MS (ESI): 429.2 ($C_{17}H_{12}Cl_2FN_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{17}H_{12}Cl_2FN_3O_3S$: C, 47.68; H, 2.82; N, 9.81. Found: C, 47.73; H, 2.89; N, 9.76.

5-Chloro-1-(4-fluorophenyl)-3-methyl-*N*-tosyl-1*H*-pyrazole-4-carboxamide (**3h**)

Light yellow solid, yield 69%; mp 160–162°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.62 (s, 1H), 8.04 (d, J=8.3 Hz, 2H), 7.48–7.41 (m, 2H), 7.37 (d, J=8.1 Hz, 2H), 7.20 (t, J=8.5 Hz, 2H), 2.47 (s, 3H), 2.45 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.16, 160.55, 150.24, 137.81, 137.63, 133.63, 132.81, 129.34, 128.37, 116.81, 116.13, 115.61, 13.72. MS (ESI): 408.8 ($C_{18}H_{15}ClFN_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{15}ClFN_3O_3S$: C, 53.01; H, 3.71; N, 10.30. Found: C, 53.09; H, 3.75; N, 10.26.

5-Chloro-1-(4-fluorophenyl)-*N*-((4-methoxyphenyl)-sulfonyl)-3-methyl-1*H*-pyrazole-4-carboxamide (**3i**)

Light yellow solid, yield 66%; mp 114–116°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.62 (s, 1H), 8.09 (d, J=8.9 Hz, 2H), 7.47–7.40 (m, 2H), 7.20 (t, J=8.4 Hz, 2H), 7.02 (d, J=8.9 Hz, 2H), 3.89 (s, 3H), 2.48 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ163.81, 163.21, 160.41, 150.23, 133.63, 133.12, 132.81, 126.12, 116.84, 116.18, 115.63, 114.63, 55.83, 13.72. MS (ESI): 424.8 ($C_{18}H_{15}ClFN_3O_4S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{15}ClFN_3O_4S$: C, 51.01; H, 3.57; N, 9.91. Found: C, 51.08; H, 3.62; N, 9.97.

5-Chloro-1-(4-fluorophenyl)-3-methyl-*N*-((4-(trifluoro-methoxy)phenyl)sulfonyl)-1*H*-pyrazole-4-carboxamide (**3j**)

Light yellow solid, yield 63%; mp 133–135°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.70 (s, 1H), 8.23 (d, J=8.8 Hz, 2H), 7.48–7.42 (m, 2H), 7.39 (d, J=8.4 Hz, 2H), 7.21 (t, J=8.4 Hz, 2H), 2.48 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.20, 160.41, 153.84, 150.20, 133.63, 133.14, 132.81, 129.74, 126.15, 116.85, 116.18, 115.67, 114.67, 13.73. MS (ESI): 478.8 ($C_{18}H_{12}ClF_4N_3O_4S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{12}ClF_4N_3O_4S$: C, 45.25; H, 2.53; N, 8.79. Found: C, 45.33; H, 2.55; N, 8.74.

5-Chloro-1-(4-chlorophenyl)-3-methyl-*N*-(phenyl-sulfonyl)-1*H*-pyrazole-4-carboxamide (**3k**)

Light yellow solid, yield 75%; mp 168–170°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.66 (s, 1H), 8.16 (d, J=7.5 Hz, 2H), 7.67 (t, J=7.5 Hz, 1H), 7.58 (t, J=7.8 Hz, 2H), 7.49 (d, J=8.7 Hz, 2H), 7.41 (d, J=8.7 Hz, 2H), 2.47 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.17, 150.21, 139.08, 138.82, 135.32, 133.63, 131.51, 129.79, 129.57, 129.45, 119.87, 116.84, 13.70. MS (ESI): 411.2 ($C_{18}H_{12}ClF_4N_3O_4S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{12}ClF_4N_3O_4S$: C, 49.77; H, 3.19; N, 10.24. Found: C, 49.80; H, 3.14; N, 10.30.

5-Chloro-1-(4-chlorophenyl)-*N*-((4-chlorophenyl)sulfonyl)-3-methyl-1*H*-pyrazole-4-carboxamide (**3l**)

Light yellow solid, yield 76%; mp 174–176°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.67 (s, 1H), 8.10 (d, J=8.6 Hz, 2H), 7.55 (d, J=8.6 Hz, 2H), 7.49 (d, J=8.7 Hz, 2H), 7.42 (d, J=8.7 Hz, 2H), 2.47 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.17, 150.27, 138.93, 137.56, 135.32, 133.70, 131.54, 129.47, 129.15, 128.76, 119.84, 116.75, 13.70. MS (ESI): 445.7 ($C_{17}H_{12}Cl_3N_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{17}H_{12}Cl_3N_3O_3S$: C, 45.91; H, 2.72; N, 9.45. Found: C, 45.97; H, 2.77; N, 9.51.

5-Chloro-1-(4-chlorophenyl)-3-methyl-*N*-tosyl-1*H*-pyrazole-4-carboxamide (**3m**)

Light yellow solid, yield 72%; mp 155–157°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.62 (s, 1H), 8.04 (d, J=8.1 Hz, 2H), 7.49 (d, J=8.6 Hz, 2H), 7.41 (d, J=8.6 Hz, 2H), 7.37 (d, J=8.1 Hz, 2H), 2.47 (s, 3H), 2.45 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.21, 150.22, 137.89, 137.65, 135.37, 133.63, 131.56, 129.45, 129.37, 128.36, 119.84, 116.81, 21.34, 13.73. MS (ESI): 425.3 ($C_{18}H_{15}Cl_2N_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{15}Cl_2N_3O_3S$: C, 50.95; H, 3.56; N, 9.90. Found: C, 50.92; H, 3.63; N, 9.96.

5-Chloro-1-(4-chlorophenyl)-*N*-((4-methoxyphenyl)-sulfonyl)-3-methyl-1*H*-pyrazole-4-carboxamide (**3n**)

Light yellow solid, yield 85%; mp 167–169°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.63 (s, 1H), 8.09 (d, J=9.0 Hz, 2H), 7.49 (d, J=8.8 Hz, 2H), 7.41 (d, J=8.8 Hz, 2H), 7.02 (d, J=9.0 Hz, 2H), 3.89 (s, 3H), 2.47 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.84, 163.24, 150.27, 135.32, 133.66, 133.12, 131.45, 129.45, 126.15, 119.84, 116.14, 114.63, 55.84, 13.76. MS (ESI): 441.3 ($C_{18}H_{15}Cl_2N_3O_4S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{15}Cl_2N_3O_4S$: C, 49.10; H, 3.43; N, 9.54. Found: C, 49.18; H, 3.48; N, 9.59.

5-Chloro-1-(4-chlorophenyl)-3-methyl-*N*-((4-(trifluoromethoxy)phenyl)sulfonyl)-1*H*-pyrazole-4-carboxamide (**3o**)

Light yellow solid, yield 86%; mp 158–160°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.69 (s, 1H), 8.23 (d, J=8.9 Hz, 2H), 7.49 (d, J=8.7 Hz, 2H), 7.40 (dd, J=13.8, 8.7 Hz, 4H), 2.48 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.26, 153.83, 150.27, 135.34, 133.71, 133.16, 131.54, 129.74, 129.45, 126.19, 119.87, 116.60, 114.67, 13.70. MS (ESI): 495.2 ($C_{18}H_{12}Cl_2F_3N_3O_4S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{12}Cl_2F_3N_3O_4S$: C, 43.74; H, 2.45; N, 8.50. Found: C, 43.78; H, 2.47; N, 8.57.

5-Chloro-3-methyl-*N*-(phenylsulfonyl)-1-(*p*-tolyl)-1*H*-pyrazole-4-carboxamide (**3p**)

Light yellow solid, yield 88%; mp 184–186°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.69 (s, 1H), 8.16 (d, J=7.4 Hz, 2H), 7.66 (t, J=7.5 Hz, 1H), 7.58 (t, J=7.8 Hz, 2H), 7.34–7.28 (m, 4H), 2.47 (s, 3H), 2.43 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.24, 150.27, 139.08, 138.82, 135.84, 134.26, 133.57, 129.79,

129.67, 129.57, 125.45, 116.59, 21.34, 13.70. MS (ESI): 390.8 ($C_{18}H_{16}ClN_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{16}ClN_3O_3S$: C, 55.46; H, 4.14; N, 10.78. Found: C, 55.52; H, 4.19; N, 10.75.

5-Chloro-*N*-((4-chlorophenyl)sulfonyl)-3-methyl-1-(*p*-tolyl)-1*H*-pyrazole-4-carboxamide (**3q**)

Light yellow solid, yield 84%; mp 114–116°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.70 (s, 1H), 8.10 (d, J=8.2 Hz, 2H), 7.54 (d, J=8.4 Hz, 3H), 7.31 (d, J=1.9 Hz, 3H), 2.47 (s, 3H), 2.43 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.17, 150.31, 138.96, 137.54, 135.94, 134.26, 133.63, 129.57, 129.16, 128.71, 125.13, 116.80, 21.33, 13.73. MS (ESI): 425.3 ($C_{18}H_{15}Cl_2N_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{18}H_{15}Cl_2N_3O_3S$: C, 50.95; H, 3.56; N, 9.90. Found: C, 51.02; H, 3.53; N, 9.98.

5-Chloro-3-methyl-1-(*p*-tolyl)-*N*-tosyl-1*H*-pyrazole-4-carboxamide (**3r**)

Light yellow solid, yield 76%; mp 80–83°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.68 (s, 1H), 8.03 (d, J=8.2 Hz, 2H), 7.36 (d, J=8.1 Hz, 3H), 7.31 (d, J=4.7 Hz, 3H), 2.47 (s, 3H), 2.45 (s, 3H), 2.43 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.20, 150.24, 137.84, 137.65, 135.94, 134.26, 133.63, 129.67, 129.31, 128.37, 125.13, 116.80, 21.37, 21.57, 13.70. MS (ESI): 404.8 ($C_{19}H_{18}ClN_3O_3S$, $[M+H]^+$). *Anal.* Calcd for $C_{19}H_{18}ClN_3O_3S$: C, 56.50; H, 4.49; N, 10.40. Found: C, 56.57; H, 4.53; N, 10.35.

5-Chloro-*N*-((4-methoxyphenyl)sulfonyl)-3-methyl-1-(*p*-tolyl)-1*H*-pyrazole-4-carboxamide (**3s**)

Light yellow solid, yield 75%; mp 89–92°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.67 (s, 1H), 8.09 (d, J=8.9 Hz, 2H), 7.31 (d, J=4.7 Hz, 3H), 7.02 (d, J=8.9 Hz, 3H), 3.88 (s, 3H), 2.47 (s, 3H), 2.42 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.84, 163.17, 150.20, 137.84, 135.99, 133.63, 133.24, 129.77, 126.19, 125.53, 116.87, 114.62, 55.86, 21.51, 13.71. MS (ESI): 420.8 ($C_{19}H_{18}ClN_3O_4S$, $[M+H]^+$). *Anal.* Calcd for $C_{19}H_{18}ClN_3O_4S$: C, 54.35; H, 4.32; N, 10.01. Found: C, 55.28; H, 4.36; N, 10.08.

5-Chloro-3-methyl-1-(*p*-tolyl)-*N*-((4-(trifluoromethoxy)-phenyl)sulfonyl)-1*H*-pyrazole-4-carboxamide (**3t**)

Light yellow solid, yield 74%; mp 129–131°C; ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 8.78 (s, 1H), 8.22 (d, J=8.7 Hz, 2H), 7.38 (d, J=8.4 Hz, 2H), 7.30 (d, J=1.6 Hz, 4H), 2.47 (s, 3H), 2.42 (s, 3H). ^{13}C-NMR (151 MHz, DMSO) δ: 163.84, 163.27, 153.83, 150.24, 135.57, 133.45, 133.14, 129.76, 129.67, 126.22, 125.13, 116.85, 114.65, 21.54, 13.72. MS (ESI): 474.8 ($C_{19}H_{15}ClF_3N_3O_4S$, $[M+H]^+$). *Anal.* Calcd for $C_{19}H_{15}ClF_3N_3O_4S$: C, 48.16; H, 3.19; N,8.87. Found: C, 48.22; H, 3.24; N, 8.91.

***In Vitro At*AHAS Inhibition** The plant *At*AHAS was expressed and purified as described previously.[22] AHAS activity was measured in accordance with the literature procedure.[23]

***In Vivo* Inhibition of the Root Growth of Rape** The *in vivo* data were determined using previously published method.[24]

Molecular Docking The crystal structures of succinate dehydrogenase (PDB entry: 5k6t) were retrieved from the Protein Data Bank. The molecular docking procedure was performed by using CDOCKER protocol for receptor–ligand interactions section of DS 3.1 (Discovery Studio 3.1; Accelrys, Inc.).[25]

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Conflict of Interest The authors declare no conflict of interest.

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