Bioorganic & Medicinal Chemistry 18 (2010) 4897–4904

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Syntheses and herbicidal activity of new triazolopyrimidine-2-sulfonamides as acetohydroxyacid synthase inhibitor

Chao-Nan Chen [a], Qiong Chen [a], Yu-Chao Liu [a], Xiao-Lei Zhu [a], Cong-Wei Niu [b], Zhen Xi [b,*], Guang-Fu Yang [a,*]

[a] Key Laboratory of Pesticide & Chemical Biology, Ministry of Education, College of Chemistry, Central China Normal University, Wuhan 430079, PR China
[b] State Key Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin 300071, PR China

ARTICLE INFO

Article history:
Received 10 May 2010
Revised 3 June 2010
Accepted 4 June 2010
Available online 11 June 2010

Keywords:
1,2,4-Triazolopyrimidine-2-sulfonamide
Acetohydroxyacid synthase inhibitor
Herbicide

ABSTRACT

The triazolopyrimidine-2-sulfonanilide, discovered from preparing bioisosteres of the sulfonylurea herbicides, is an important class of acetohydroxyacid synthase (AHAS, EC 4.1.3.18) inhibitors. At least over ten triazolopyrimidine sulfonanilides have been commercialized as herbicides for the control of broadleaf weeds and grass with cereal crop selectivity. Herein, a series of triazolopyrimidine-2-sulfonanilides were designed and synthesized with the aim of discovery of new herbicides with higher activity. The assay results of the inhibition activity of the synthesized compounds against *Arabidopsis thatiana* AHAS indicated that some compounds showed a little higher activity against flumetsulam (FS), the first commercial triazolopyrimidine-2-sulfonanilide-type herbicide. The k_i values of two promising compounds **3d** and **8h** are respectively, 1.61 and 1.29 μM, while that of FS is 1.85 μM. Computational simulation results indicated the ester group of compound **3d** formed hydrogen bonds with the surrounding residues Arg'198 and Ser653, which accounts for its 11.5-folds higher AHAS inhibition activity than Y6610. Further green house assay showed that compound **3d** has comparable herbicidal activity as FS. Even at the concentration of 37.5 g.ai/ha, **3d** showed excellent herbicidal activity against *Galium aparine*, *Cerastium arvense*, *Chenopodium album*, *Amaranthus retroflexus*, and *Rμmex acetasa*, moderate herbicidal activity against *Polygonum humifusum*, *Cyperus iria*, and *Eclipta prostrate*. The combination of in vitro and in vivo assay indicated that **3d** could be regarded as a new potential acetohydroxyacid synthase-inhibiting herbicide candidate for further study.

1. Introduction

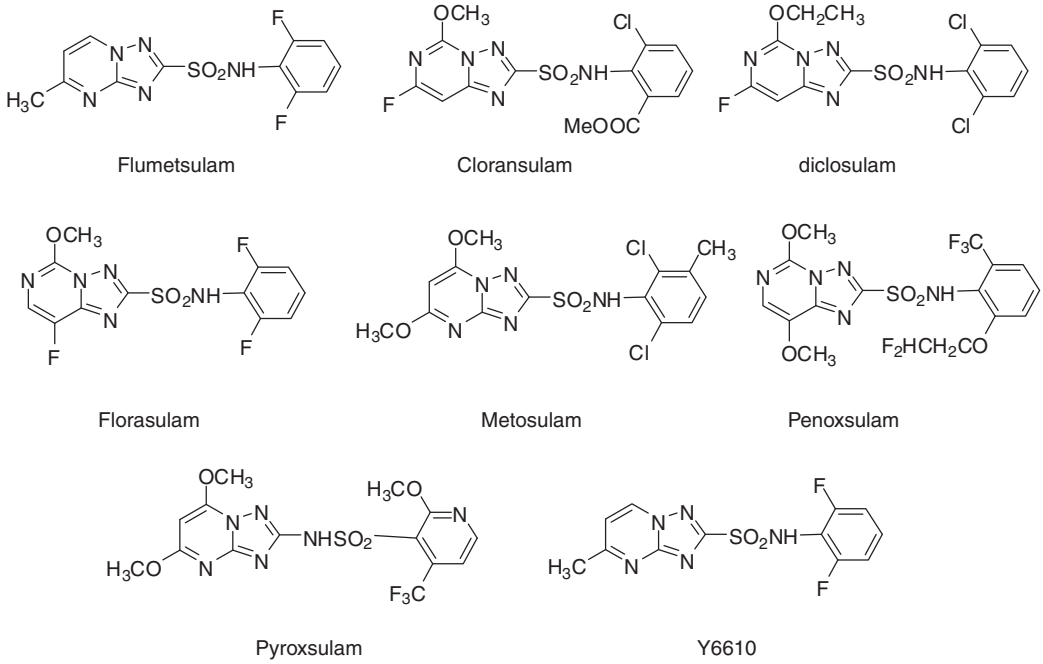
Triazolopyrimidine-2-sulfonamide is one of the most important herbicidal groups targeting acetohydroxyacid synthase (AHAS, also known as acetolactate synthase; EC 2.2.1.6, formerly EC 4.1.3.18), which is known to catalyze the biosynthesis of branched-chain amino acids including valine, leucine, and isoleucine.[1–10] It has been demonstrated that triazolopyrimidine-2-sulfonamides competed with the amino acid leucine for binding to AHAS isolated from cotton (*Gossypium hirsutum*).[11] Up to now, at least seven 1,2,4-triazolopyrimidine-2-sulfonamide herbicides (Scheme 1) are commercially available, including flumetsulam (FS),[4] penoxsulam,[5] cloransulam,[6] metosulam,[7] florasulam,[8] diclosulam,[9] and pyroxsulam,[10] among which FS, *N*-2,6-difluorophenyl-5-methyl-1,2,4-triazolo[1,5-*a*]pyrimidine-2-sulfonamide, is the first product extensively adopted in the control of broad-leaf weeds and grasses in soya beans, peas and maize field. However, because of its high herbicidal activity on broad-leaf plants and slow degradation rate, the trace residues of FS in soil will have adverse effect on the quality and yield of following crops.[12,13]

Previously, with the aim to discover new triazolopyrimidine-2-sulfonamide compounds with high herbicidal activity and faster degradation rate in soil, we designed and synthesized a new herbicidal compound, *N*-2,6-difluorophenyl-5-methoxy-1,2,4-triazolo[1,5-*a*]pyrimidine-2-sulfonamide (experimental code: Y6610, Scheme 1).[14] Because of the introduction of methoxy group rather than the methyl group in FS, compound Y6610 had shorter half-life in soil than FS. However, the enzymatic kinetic results indicated that compound Y6610 has about 10-fold lower enzyme-inhibiting activity ($k_i = 3.31 \times 10^{-6}$ M, against *Arabidopsis thaliana* AHAS) than FS, whose k_i values against *A. thaliana* AHAS is 3.60×10^{-7} M.

Herein, we reported the further structural optimization of Y6610 and the synthesis of 22 new triazolopyrimidine-2-sulfonamides. The in vitro enzyme inhibition and in vivo herbicidal activities assay indicated that some compounds displayed higher enzyme inhibition activity than Y6610 and FS. Most interestingly, these compounds with high enzyme inhibition activity also displayed excellent broad spectrum herbicidal activities at the dosage of 37.5–150 g/ha.

* Corresponding author. Tel.: +86 27 67867800; fax: +86 27 67867141.
E-mail address: gfyang@mail.ccnu.edu.cn (G.-F. Yang).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.06.015



Scheme 1. Chemical structure of commercial triazolopyrimidine herbicides and Y6610.

2. Materials and methods

Unless otherwise noted, reagents were purchased from commercial suppliers and used without further purification, as all solvents were redistilled before use. ^{1}H NMR spectra were recorded on a Mercury-Plus 400 spectrometer in $CDCl_3$ or DMSO with TMS as the internal reference. MS spectra were determined using a TraceMS 2000 organic mass spectrometer. Elemental analyses were performed on a Vario EL III elemental analysis instrument. Melting points were measured on a Buchi B-545 melting point apparatus and are uncorrected. Intermediates **1** and **4** were synthesized according to the existing methods.[14–16]

2.1. General procedure for the synthesis of compound 3

Chlorine was bubbled into a suspension of **1** (40 mmol) in 100 mL of methylene chloride and 50 mL of water cooled to −1 °C. During the course of the addition the temperature of the reaction mixture was maintained below 5 °C. After the reaction finished by TLC detection, the organic layer was separated and the aqueous layer was extracted twice with methylene chloride. The combined organic phases were dried with $MgSO_4$ and evaporated at reduced pressure to obtain crude product, which was used directly for the next step reaction without further purification.

The starting substituted aniline (60 mmol) was dissolved in 100 mL of pyridine. Then, the above-obtained crude product in 100 mL of dichloromethane was added. The resulted reaction mixture was stirred at room temperature and monitored by TLC. After the reaction finished, the pyridine was removed by evaporation at reduced pressure and the residue was treated with 200 mL of NaOH (0.5 M) and 100 mL of dichloromethane. After filteration, the aqueous layer was separated and acidified with HCl (3 M). The precipitate which separated upon acidification was collected by filtration and dried to give 5.2 g (25–40% overall yield from **1**) of the desired product as a yellow solid.

2.1.1. Date for 3a

Yield: 30.2%; mp 211–213 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.04 (s, 3H, OCH_3), 7.05 (d, 1H, J = 7.8 Hz, Het-H), 7.26–7.32 (m, 2H, Ar-H), 9.23 (d, 1H, J = 7.2 Hz, Het-H), 11.16 (s, 1H, NH); EI MS: m/z (%) 360 ([M+1], 100), 359 (M^+, 38.2), 358 ([M−1], 56.2), 276 (35.3), 120 (37.8), 100 (65.5), 91 (6.2). Anal. Calcd for $C_{12}H_8F_3N_5O_3S$: C, 40.12; H, 2.24; N, 19.49. Found: C, 40.39; H, 2.49; N, 19.74.

2.1.2. Date for 3b

Yeild: 40%; mp 206–207 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.04 (s, 3H, OCH_3), 7.05 (d, 1H, J = 7.2 Hz, Het-H), 7.09–7.34 (m, 3H, Ar-H), 9.25 (d, 1H, J = 7.8 Hz, Het-H), 11.16 (s, 1H, NH); EI MS: m/z (%) 342 ([M+1], 100), 341 (M^+, 24.3), 340 ([M−1], 76.0), 276 (28.6), 257 (49.7), 149 (38.8), 126 (33.7), 120 (50.8), 100 (43.5), 91 (6.2). Anal. Calcd for $C_{12}H_9F_2N_5O_3S$: C, 42.23; H, 2.66; N, 20.52. Found: C, 42.24; H, 2.70; N, 20.35.

2.1.3. Date for 3c

Yeild: 30.8%; mp 217–218 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.03 (s, 3H, OCH_3), 7.04 (d, 1H, J = 7.8 Hz, Het-H), 7.14 (t, 1H, J = 7.8 Hz, Ar-H), 7.09–7.34 (m, 2H, Ar-H), 7.40 (t, 1H, J = 7.8 Hz, Ar-H), 9.23 (d, 1H, J = 7.8 Hz, Het-H), 10.86 (s, 1H, NH); EI MS: m/z (%) 323 (M, 100), 322 (95.9), 258 (45.9), 239 (64.2), 150 (53.7), 121 (14.6), 110 (67.4), 108 (80.1), 91 (6.3), 77 (6.3). Anal. Calcd for $C_{12}H_{10}FN_5O_3S$: C, 44.58; H, 3.12; N, 21.66. Found: C, 44.93; H, 3.14; N, 21.20.

2.1.4. Date for 3d

Yeild: 33.8%; mp 224–225 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 3.67 (s, 3H, $COOCH_3$), 4.03 (s, 3H, OCH_3), 7.03 (d, 1H, J = 7.8 Hz, Het-H), 7.46 (t, 1H, J = 7.8 Hz, Ar-H), 7.68–7.71 (m, 2H, Ar-H), 9.22 (d, 1H, J = 7.8 Hz, Het-H), 10.82 (s, 1H, NH); EI MS: m/z (%) 341 (100), 340 (95.7), 257 (63.9), 148 (51.5), 127 (42.6), 120 (30.6), 100 (79.8), 91 (11.3). Anal. Calcd for $C_{12}H_{10}FN_5O_3S$: C, 42.27; H, 3.04; N, 17.61. Found: C, 42.07; H, 3.12; N, 17.26.

2.1.5. Date for 3e

Yeild: 47.0%; mp 280–281 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.01 (s, 3H, OCH_3), 7.03 (d, 1H, J = 7.8 Hz, Het-H), 7.20 (d, 2H, J = 8.4 Hz, Ar-H), 7.48 (d, 2H, J = 8.4 Hz, Ar-H), 9.22 (d, 1H, J = 7.8 Hz, Het-H), 11.22 (s, 1H, NH); EI MS: *m/z* (%) 385 ([M+1], 90.4), 384 (M^+, 100), 383 ([M−1], 99.3), 382 (81.7), 319 (30.5), 240 (26.0), 171 (64.5), 91 (31.3). Anal. Calcd for $C_{12}H_{10}BrN_5O_3S$: C, 37.51; H, 2.62; N, 18.23. Found: C, 37.44; H, 2.76; N, 18.45.

2.1.6. Date for 3f

Yeild: 30.7%; mp 187–188 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.04 (s, 3H, OCH_3), 7.06 (d, 1H, J = 7.8 Hz, Het-H), 7.35 (t, 1H, J = 8.4 Hz, Ar-H), 7.45 (d, 1H, J = 7.8 Hz, Ar-H), 7.56 (d, 1H, J = 8.4 Hz, Ar-H), 9.25 (d, 1H, J = 7.2 Hz, Het-H), 10.99 (s, 1H, NH); EI MS: *m/z* (%) 374 (M^+, 7.88), 373 (61.2), 372 (51.7), 323 (72.6), 322 (100), 259 (27.3), 258 (44.2), 149 (30.4), 109 (46.3), 91 (12), 77 (8.3). Anal. Calcd for $C_{12}H_9Cl_2N_5O_3S$: C, 38.52; H, 2.42; N, 18.72. Found: C, 38.41; H, 2.04; N, 18.20.

2.1.7. Date for 3g

Yeild: 34.0%; mp 177–178 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.04 (s, 3H, OCH_3), 7.05 (d, 1H, J = 7.8 Hz, Het-H), 7.22 (t, 1H, J = 7.8 Hz, Ar-H), 7.35 (t, 1H, J = 7.8 Hz, Ar-H), 7.41 (d, 1H, J = 7.2 Hz, Ar-H), 7.65 (d, 1H, J = 7.2 Hz, Ar-H), 9.24 (d, 1H, J = 7.8 Hz, Het-H), 10.67 (s, 1H, NH); EI MS: *m/z* (%) 385 ([M+1], 10.9), 384 (M^+, 19.3), 383 ([M−1], 16.3), 304 (88.7), 303 (100), 239 (9.3), 197 (15.9), 169 (18.8), 108 (12.3), 91 (20.8). Anal. Calcd for $C_{12}H_{10}BrN_5O_3S$: C, 37.51; H, 2.62; N, 18.23. Found: C, 37.81; H, 2.75; N, 18.49.

2.1.8. Date for 3h

Yeild: 28.5%; mp 222–223 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 4.01 (s, 3H, OCH_3), 7.03 (d, 1H, J = 7.2 Hz, Het-H), 7.32–7.33 (m, 4H, Ar-H), 9.22 (d, 1H, J = 7.2 Hz, Het-H), 11.28 (s, 1H, NH); EI MS: *m/z* (%) 390 ([M+1], 26.0), 389 (M^+, 76.4), 388 ([M−1], 100), 324 (49.2), 255 (29.2), 175 (48.3), 148 (40.9), 91 (4.3), 77 (7.3). Anal. Calcd for $C_{13}H_{10}F_3N_5O_4S$: C, 40.11; H, 2.59; N, 17.99. Found: C, 40.39; H, 2.82; N, 18.02.

2.2. General procedure for the synthesis of intermediates 5

Compound **4** (0.086 mol), 15.5 mL of carbon disulfide (0.258 mol), 48 mL of triethylamine (0.344 mol), and 400 mL of ethanol were mixed and heated to reflux with stirring for 2.5 h. After the resulted mixture was cooled to ambient temperature, 16.4 g of benzyl chloride (0.129 mol) was then added with stirring. The mixture was allowed to react for additional 3 h. The volatiles were removed by evaporation under reduced pressure and the residue was dissolved in methylene chloride. The resulted solution was washed with water, dried over magnesium sulfate and concentrated under reduced pressure. The residue was triturated with hexane and filtered to obtain the crude product **5** (mixed with trace **6**). The obtained product was used for next reaction without further purification.

2.3. General procedure for the synthesis of intermediates 6

A solution of sodium methoxide in methanol (7.4 mL, 0.033 mol) was added to a solution of 19.9 g of **5** (~0.065 mol, containing trace of **6**) in 200 mL methanol at ambient temperature with stirring and allowed to react for about 30 min. Then, acetic acid (10 mL) was added and the volatiles were removed by evaporation under reduced pressure. The residue was dissolved in methylene chloride and the resulting solution was washed with water, dried over magnesium sulfate, and concentrated in vacuo. The residue was triturated with hexane, filtered, and dried to obtain the desired compounds **6**.

2.3.1. Date for 6a

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.30 (s, 3H), 2.63 (s, 3H), 4.55 (s, 2H), 6.89 (s, 1H), 7.22 (m, 1H), 7.35 (d, J = 7.8 Hz, 2H), 7.44 (d, J = 7.8 Hz, 2H).

2.3.2. Date for 6b

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.68 (s, 3H), 4.55 (s, 2H), 6.93 (s, 1H), 7.22 (m, 1H), 7.35 (d, J = 7.8 Hz, 2H), 7.46 (d, J = 7.8 Hz, 2H).

2.3.3. Date for 6c

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H), 2.63 (s, 3H), 4.55 (s, 2H), 7.24 (m, 1H), 7.33 (d, J = 7.8 Hz, 2H), 7.44 (d, J = 7.8 Hz, 2H).

2.4. General procedure for the synthesis of compound 8

Chlorine was bubbled into a suspension of **6** (40 mmol) in 100 mL of methylene chloride, 50 mL of water cooled to −1 °C. During the course of the addition the temperature of the reaction mixture was kept under 5 °C. After the reaction finished by TLC detection, the organic layer was separated and the aqueous layer was extracted twice with methylene chloride. The combined organic phases were dried ($MgSO_4$) and evaporated at reduced pressure to obtain crude product **7**, which was used for he next reaction without further purification.

The starting substituted alinine (60 mmol) was dissolved in 100 mL of pyridine and the above suspension in 100 mL dichloromethane was added. The reaction mixture was stirred at room temperature and monitored by TLC. The pyridine was removed by evaporation at reduced pressure after the reaction finished, and the residue was treated with 200 mL of NaOH (0.5 M) and dichloromethane (100 mL). The aqueous layer was separated by filtration and acidified with 3 M HCl. The precipitate which separated upon acidification was collected by filtration and dried to give 5.2 g (25–40% overall yield from **6**) of the desired product as a yellow solid.

2.4.1. Date for 8a

Yeild: 44.7%; mp 140–141 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.61 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 7.21–7.30 (m, 2H, Ar-H)), 11.25 (s, 1H, NH); EI MS: *m/z* (%) 393 ([M+2], 31), 391 (M^+, 95), 367 (48), 145 (100), 119 (40). Anal. Calcd for $C_{13}H_9ClF_3N_5O_2S$: C, 39.86; H, 2.32; N, 17.88. Found: C, 39.54; H, 2.55; N, 17.42.

2.4.2. Date for 8b

Yeild: 30.5%; mp 148–149 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.12 (s, 3H, CH_3), 6.90–7.55 (m, 3H, Ar-H), 7.56 (s, 1H, Het-H), 8.05 (s, 1H, NH); EI MS: *m/z* (%) 393 (M^+, 44), 265 (28), 161 (81), 152 (23), 149 (33), 128 (37), 101 (88), 64 (40), 54 (60), 51 (100). Anal. Calcd for $C_{13}H_8F_5N_5O_2S$: C, 39.70; H, 2.05; N, 17.81. Found: C, 40.13; H, 2.36; N, 17.39.

2.4.3. Date for 8c

Yeild: 29.5%; mp 185–186 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.66 (s, 3H, CH_3), 2.92 (s, 3H, CH_3), 7.03–7.54 (m, 4H, Ar-H)), 7.71 (s, 1H, NH); EI MS: *m/z* (%) 357 ([M+2], 28), 355 (M^+, 100), 291 (22), 110 (49), 83 (28). Anal. Calcd for $C_{13}H_{11}ClFN_5O_2S$: C, 43.89; H, 3.12; N, 19.68. Found: C, 44.16; H, 3.59; N, 19.28.

2.4.4. Date for 8d

Yeild: 27.2%; mp 212–213 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.51 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 7.22–7.30 (m, 2H, Ar-H), 7.75 (s, 1H, Het-H), 11.13 (s, 1H, NH); EI MS: *m/z* (%) 357 (M^+, 100), 274 (27), 146 (51). Anal. Calcd for $C_{13}H_{10}F_3N_5O_2S$: C, 43.70; H, 2.82; N, 19.60. Found: C, 43.37; H, 2.99; N, 19.32.

2.4.5. Date for 8e

Yeild: 25.0%; mp 163–164 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.09 (s, 3H, CH_3), 6.96–7.47 (m, 2H, Ar-H), 7.60 (s, 1H, Het-H), 8.06 (s, 1H, NH); EI MS: *m/z* (%) 411 (M^+, 91), 391 (69), 390 (53), 318 (44), 290 (24), 261 (24), 209 (100), 161 (22), 146 (99), 101 (96), 91 (36), 72 (36), 70 (21), 45 (24), 42 (43). Anal. Calcd for $C_{13}H_7F_6N_5O_2S$: C, 37.96; H, 1.72; N, 17.03. Found: C, 38.06; H, 1.65; N, 17.50.

2.4.6. Date for 8f

Yeild: 46.6%; mp 233–234 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.61 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 7.32–7.67 (m, 2H, Ar-H), 7.68 (m, 2H, Ar-H), 11.79 (s, 1H, NH); EI MS: *m/z* (%) 407 ([M+2], 4), 405 (M^+, 9), 214 (10), 182 (13), 141 (12), 125 (11), 91 (100). Anal. Calcd for $C_{14}H_{11}ClF_3N_5O_2S$: C, 41.44; H, 2.73; N, 17.26. Found: C, 41.63; H, 2.46; N, 17.62.

2.4.7. Date for 8g

Yeild: 37.1%; mp 176–177 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.53 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 7.12–7.40 (m, 4H, Ar-H), 7.75 (s, 1H, Het-H), 10.96 (s, 1H, NH); EI MS: *m/z* (%) 321 (M^+, 100), 257 (14), 109 (13). Anal. Calcd for $C_{13}H_{12}FN_5O_2S$: C, 48.59; H, 3.76; N, 21.79. Found: C, 48.81; H, 3.94; N, 21.96.

2.4.8. Date for 8h

Yeild: 39.6%; Mp 209–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.65 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 3.64 (s, 3H, CH_3), 7.46–7.72 (m, 3H, Ar-H), 11.09 (s, 1H, NH); EI MS: *m/z* (%) 431 ($[M+2]^+$, 23), 430 ($[M+1]^+$, 35), 405 (M^+, 69), 394 (100), 362 (36), 333 (33), 308 (29), 153 (29). Anal. Calcd for $C_{15}H_{13}Cl_2N_5O_4S$: C, 41.87; H, 3.05; N, 16.28. Found: C, 42.20; H, 2.92; N, 16.26.

2.4.9. Date for 8i

Yeild: 43.5%; mp 151–152 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.96 (s, 3H, CH_3), 7.13–7.36 (m, 4H, Ar-H), 8.64 (s, 1H, Het-H), 11.12 (s, 1H, NH); EI MS: *m/z* (%) 376 ($[M+1]^+$, 11), 375 (M^+, 100), 110 (70), 82 (30). Anal. Calcd for $C_{13}H_9F_4N_5O_2S$: C, 41.60; H, 2.42; N, 18.66. Found: C, 41.99; H, 2.35; N, 18.97.

2.4.10. Date for 8j

Yeild: 26.4%; mp 241–242 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.97 (s, 3H, CH_3), 7.36–7.53 (m, 3H, Ar-H), 8.65 (s, 1H, Het-H), 11.20 (s, 1H, NH); EI MS: *m/z* (%) 426 ($[M+1]^+$, 11), 425 (M^+, 33), 392 (84), 390 (96), 389 (100), 162 (40), 160 (82). Anal. Calcd for $C_{13}H_8Cl_2F_3N_5O_2S$: C, 36.64; H, 1.89; N, 16.43. Found: C, 36.99; H, 1.53; N, 16.78.

2.4.11. Date for 8k

Yeild: 26.1%; mp 148–149 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.11 (s, 3H, CH_3), 6.80–8.03 (m, 3H, Ar-H), 7.58 (s, 1H, Het-H), 8.04 (s, 1H, NH); EI MS: *m/z* (%) 393 (M^+, 100), 308 (22), 161 (22), 128 (61), 101 (28). Anal. Calcd for $C_{13}H_8F_5N_5O_2S$: C, 39.70; H, 2.05; N, 17.81. Found: C, 40.12; H, 1.97; N, 18.25.

2.4.12. Date for 8l

Yeild: 25.5%; mp 165–166 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.60 (s, 3H, CH_3), 2.97 (s, 3H, CH_3), 7.41 (s, 1H, Het-H), 7.51 (d, 1H, J = 8.4 Hz, Ar-H), 7.62 (s, 1H, Ar-H), 7.51 (d, 1H, J = 9.0 Hz, Ar-H), 10.04 (s, 1H, NH); EI MS: *m/z* (%) 406 ([M+1], 12.5), 405 (M^+, 25.4), 404 ([M−1], 22.5), 370 (100), 369 (87.0), 305 (14.9), 195 (24.3), 148 (23.0), 106 (29.6), 91 (5.85). Anal. Calcd for $C_{14}H_{11}ClF_3N_5O_2S$: C, 41.44; H, 2.73; N, 17.26. Found: C, 41.12; H, 2.67; N, 17.05.

2.4.13. Date for 8m

Yeild: 29.2%; mp 178–179 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.19 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 7.21–7.25 (m, 2H, Ar-H), 7.36 (s, 1H, Ar-H), 7.76 (s, 1H, Het-H), 10.69 (s, 1H, NH); EI MS: *m/z* (%) 351 (M^+, 5.0), 307 (9.4), 237 (9.3), 148 (48.7), 141 (61.5), 140 (100), 139 (67.3), 117 (42.2), 105 (89.5), 91 (25.3), 77 (71.0). Anal. Calcd for $C_{14}H_{14}ClN_5O_2S$: C, 47.80; H, 4.01; N, 19.91. Found: C, 48.09; H, 4.31; N, 19.80.

2.4.14. Date for 8n

Yeild: 27.4%; mp 177–178 °C; ^{1}H NMR (600 MHz, DMSO-d_6): δ 2.61 (s, 3H, CH_3), 2.86 (s, 3H, CH_3), 7.11–7.33 (m, 3H, Ar-H), 7.75 (s, 1H, Het-H), 11.28 (s, 1H, NH); EI MS: *m/z* (%) 340 ([M+1], 23.6), 339 (M^+, 98.7), 338 ([M−1], 70.5), 274 (41.8), 255 (51.2), 181 (27.7), 148 (67.3), 127 (100), 100 (31.3), 69 (21.2), 57 (31.9). Anal. Calcd for $C_{13}H_{11}F_2N_5O_2S$: C, 46.02; H, 3.27; N, 20.64. Found: C, 46.25; H, 3.49; N, 20.37.

2.4.15. X-ray diffraction analysis of 8e

Colorless blocks of **8e** ($0.20 \times 0.10 \times 0.10$ mm) were counted on a quartz fiber with protection oil. Cell dimensions and intensities were measured at 298 ± 2 K on a Bruker SMART CCD area detector diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å); θ_{max} = 25.99; 12085 measured reflections; 3112 independent reflections (R_{int} = 0.0700) of which 2508 had $I > 2\sigma(I)$. Data were corrected for Lorentz and polarization effects and for absorption (T_{min} = 0.8959; T_{max} = 0.9944). The structure was solved by direct methods using SHELXS-2001,[17] all other calculations were performed with Bruker SAINT System and Bruker SMART programs.[18] Full-matrix least-squares refinement based on F^2 using the weight of $1/[\sigma^2(F_o^2) + (0.0849P)^2 + 0.0555P]$ gave final values of R = 0.0654, ωR = 0.1697, and GOF(F) = 0.583. Hydrogen atoms were observed and refined with a fixed value of their isotropic displacement parameter.

2.5. Determination of k_i value against *A. thaliana* AHAS

he expression and purification of wild-type *A. thaliana* AHAS was performed as described in existing method.[19,20] During purification of AHAS, the inhibitory activities of the synthesized compounds against wild-type *A. thaliana* AHAS activity was assayed using the colorimetric method of Singh et al. exactly as described previously.[20] The optimized assay contained pyruvate (100 mM), ThDP (1 mM), $MgCl_2$ (10 mM), and FAD (10 μM) in 50 mM of potassium phosphate buffer at pH 7.5. The DMSO solutions of different concentrations of compounds were used for the assay. The final amount of DMSO in the assay mixture was kept under four percent so that the effect of DMSO on the reaction can be ignored. After pre-incubation at room temperature for 5 min, the reaction was initiated by the addition of AHAS enzyme. Then, after incubation at 37 °C for 1 h, the reaction was stopped by the addition of sufficient H_2SO_4 (3 M). Upon incubation at 60 °C for 15 min, the enzymatic product of AHAS activity, 2-acetolactate, is converted to acetoin. Acetoin was then quantified by further incubation at 60 °C for 15 min in the presence of 0.5% creatine and 0.5% α-naphthol. The k_i^{app} values were determined by fitting the data to equation:

$$v_i = v_\infty + (v_0 - v_\infty)/(1 + [I]/k_i^{app})$$

In this equation, v_i and v_0 represent the rates in the presence or absence of the test compound, respectively, and [I] is the concentration of the compound. The k_i^{app} is apparent inhibition constant, that is, the concentration of inhibitor giving 50% inhibition. If the initial analysis indicated that the residual activity (v_∞) at a saturating inhibitor concentration is not significantly greater than zero, the data reanalyzed with the $v_\infty = 0$. k_i^{app} was calculated by nonlinear least-squares and the Simplex method for error minimization.

2.6. Herbicidal activities assay

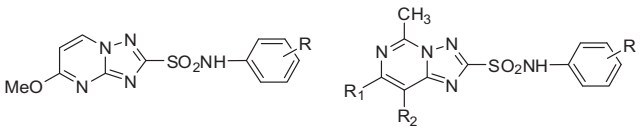
The herbicidal activities of all target compounds against *Echinochloa crusgalli, Digiatria sanguinalis, Poa annua, Brassica juncea, Amaranthus retroflexus, Eclipta prostrate, Galium aparine, Cerastium arvense, Polygonum humifusum, Cyperus iria, Chenopodium album,* and *Rμmex acetasa* were evaluated according to a previously reported procedure,[14,21–23] Y6610 and FS were selected as a control. All test compounds were formulated as a 100 g/L emulsified concentrates by using DMF as solvent and TW-80 as emulsification reagent. The concentrates were diluted with water to the required concentration and applied to pot-grown plants in a greenhouse. The soil used was a clay soil, pH 6.5, 1.6% organic matter, 37.3% clay particles, and CEC 12.1 mol/kg. The rate of application (g.ai/ha) was calculated by the total amount of active ingredient in the formulation divided by the surface area of the pot. Plastic pots with a diameter of 9.5 cm were filled with soil to a depth of 8 cm. Approximately 20 seeds of *E. crusgalli, D. sanguinalis, P. annua, B. juncea, A. retroflexus, E. prostrate, G. aparine, C. arvense, P. humifusum, C. iria, C. album* and *R. acetasa* were sown in the soil at the depth of 1–3 cm and grown at 15–30 °C in a greenhouse. The diluted formulation solutions were applied for post-emergence treatment, dicotyledon weeds were treated at the 2-leaf stage and monocotyledon weeds were treated at the 1-leaf stage, respectively. The post-emergence application rate was 150 g.ai/ha. Untreated seedlings were used as the control group and the solvent (DMF) treated seedlings were used as the solvent control group. Herbicidal activity was evaluated visually 15 days post treatment. The results of herbicidal activities were shown in Tables 1 and 2.

3. Results and discussion

3.1. Synthetic chemistry of the title compounds

All the title compounds were synthesized according to two different routes showed in Scheme 2. In route A, the starting material **1**, prepared readily from 3-amino-5-benzylthio-1,2,4-triazole according to the method as described previously,[14] was chlorosulfonylated by chlorine to afford 5-methoxy-1,2,4-triazolo[1,5-*a*]pyrimidine-2-sulfonyl chloride **2**. Without purification, compound **2** reacted with various aniline to afford the desired compounds **3a**–**h** in overall yields of 25–40% from compound **1**. In route B, cyclization of **4** with carbon disulfide afforded 3-benzylthio-5,7,8-trisubstituted-1,2,4-triazolo[4,3-*c*]pyrimidines **5**, which was rearranged in the presence of sodium methoxide to the corresponding 2-benzylthio-1,2,4-triazolo[1,5-*c*]pyrimidines **6**. Then, **6** reacted with chlorine gas under ice bath to afford the key intermediate **7**, which reacted subsequently with substituted anilines using pyridine as base to yield the target compounds **8a**–**n** smoothly in overall yields of 25–45%. The structures of all intermediates and target compounds were characterized by ^{1}H NMR, EI-MS spectral data and elemental analyses. In addition, the crystal structure of **8e** was determined by X-ray diffraction analysis. As shown in Figure 1, the fused triazolopyrimidine system

Table 1
AHAS inhibition activities and herbicidal activities of compounds **3a**–**h** and **8a**–**n**



3a~h **8**a~n

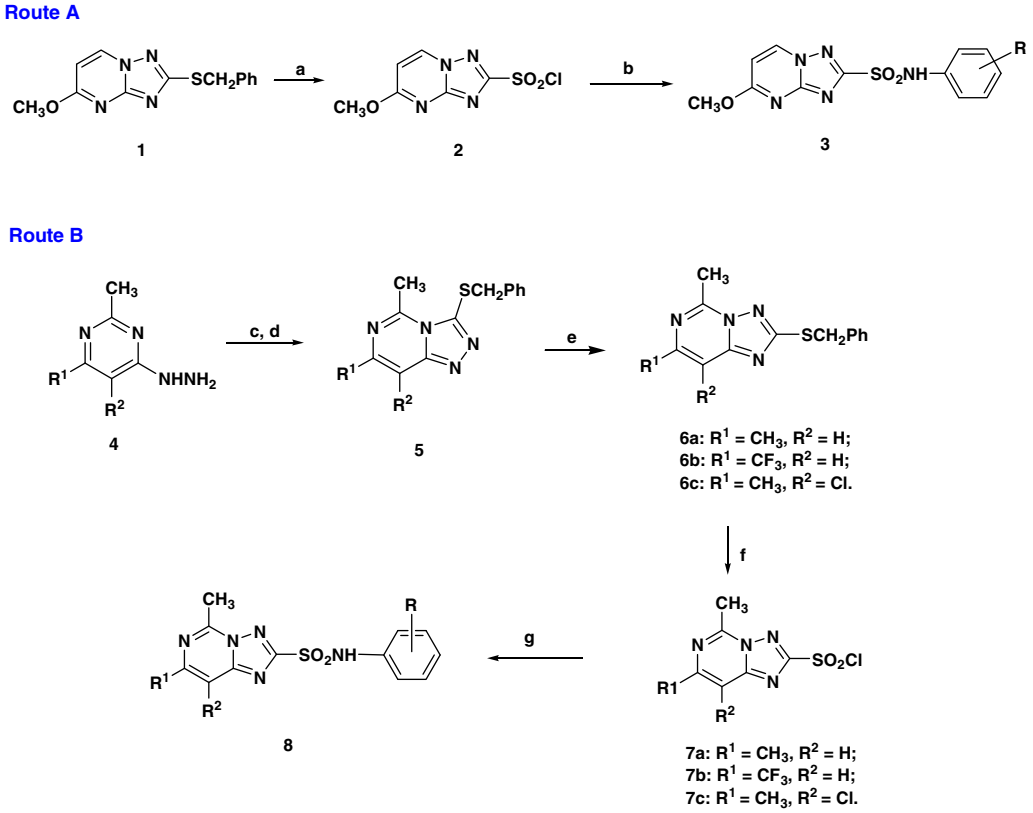
No.	R^1	R^2	R	k_i[a] (μM)	Post-emergence[b] (150 g.ai/ha)					
					EC[c]	DS	PA	BJ	AR	EP
3a	/	/	3,4,5-F_3	>1000	–	–	–	–	–	–
3b	/	/	2,5-F_2	53.3	–	–	–	+	+	+
3c	/	/	2-F	25.9	–	–	–	+	+	**++**
3d	/	/	2-Cl-6-COOMe	1.61	+	–	–	**+++**	**+++**	**+++**
3e	/	/	4-Br	>1000	–	–	–	–	–	–
3f	/	/	2,3-Cl_2	14.8	–	–	–	**++**	+	**++**
3g	/	/	2-Br	22.6	–	–	–	**++**	+	**++**
3h	/	/	4-OCF_3	>1000	–	–	–	–	–	–
8a	CH_3	Cl	2,3,4-F_3	>1000	–	–	–	–	–	–
8b	CF_3	H	2,6-F_2	70.6	–	–	–	**++**	+	**++**
8c	CH_3	Cl	2-F	25.8	–	–	–	+	+	+
8d	CH_3	H	2,3,4-F_3	>1000	–	–	–	–	–	+
8e	CF_3	H	2,3,4-F_3	>1000	–	–	–	–	–	+
8f	CH_3	Cl	4-CF_3	>1000	–	–	–	–	+	+
8g	CH_3	H	2-F	27.7	–	–	–	+	+	+
8h	CH_3	Cl	2-Cl-6-COOMe	1.29	–	–	–	**+++**	+	**++**
8i	CF_3	H	2-F	569	–	–	–	+	+	+
8j	CF_3	H	2,6-Cl_2	5.02	–	–	–	**+++**	+	**+++**
8k	CF_3	H	2,5-F_2	ND[d]	–	–	–	–	–	–
8l	CH_3	H	2-Cl-4-CF_3	178	–	–	–	–	–	–
8m	CH_3	H	2-CH_3-5-Cl	7.75	–	–	–	–	–	+
8n	CH_3	H	2,5-F_2	28.1	–	–	–	–	–	–
Y6610	/	/	/	18.5	–	–	ND	+++	+++	+++
FS	/	/	/	1.85	+	++	+	+++	+++	+++

[a] The k_i values were determined against wild-type *A. thaliana* AHAS (EC 4.1.3.18).
[b] EC for *E. crusgalli*, DS for *D. sanguinalis*, PA for *P. annua*, BJ for *B. juncea*, AR for *A. retroflexus*, and EP for *E. prostrate*.
[c] Rating system for the growth inhibition percentage: +++ ⩾ 90%; ++ ⩾ 80%; +, 50–80%; –, <50%.
[d] Not determined.

Table 2
Herbicidal spectrum of selected compounds (post-emergence)

No.	Dosage[b] (g.ai/ha)	GA[a]	PH	CAR	CI	CA	AR	RA	EP
3d	37.5	++	+	++	+	++	++	++	+
	75	+++	+	++	+	+++	++	++	++
	150	+++	+	+++	+	+++	++	++	+++
3f	37.5	+	+	–	+	–	+	+	+
	75	+	+	–	+	–	+	+	+
	150	+	+	–	+	–	++	+	+
3j	37.5	++	+	–	–	+	+	+	–
	75	++	+	–	–	+	+	+	–
	150	++	+	+	–	+	++	+	–
8b	37.5	+	–	+	–	++	–	+	+
	75	++	–	+	–	++	–	+	++
	150	++	+	+	–	+++	–	+	++
8h	37.5	++	+	–	+	++	+	+	+
	75	++	+	+	+	++	+	+	+
	150	++	+	+	+	++	+	+	+
8j	37.5	+	+	–	+	++	–	++	+
	75	++	++	+	+	++	–	++	+
	150	++	++	+	+	+++	–	++	+++
FS	37.5	+	++	+++	+	+	+++	+	+++
	75	+	+++	+++	+	+++	+++	+	+++
	150	++	+++	+++	+	+++	+++	++	+++

[a] GA for *G. aparine*, PH for *P. humifusum*, CAR for *C. arvense*, CI for *C. iria*, CA for *C. album*, AR for *A. retroflexus*, RA for *R. acetasa*, and EP for *E. prostrate*.
[b] Rating system for the growth inhibition percentage: +++ ≥ 90%;++ ≥ 80%; +, 50–80%; –, <50%.



a: Cl_2, CH_2Cl_2/H_2O, 0-5ºC; *b*: RNH_2, Py, CH_2Cl_2, *rt*; *c*: CS_2 , NEt_3, EtOH, reflux; *d*: $PhCH_2Br$, *rt*; *e*: CH_3ONa, CH_3OH, *rt*; *f*: Cl_2, CH_2Cl_2/H_2O, 0-5ºC; *g*: RNH_2, Py, CH_2Cl_2, *rt*.

Scheme 2.

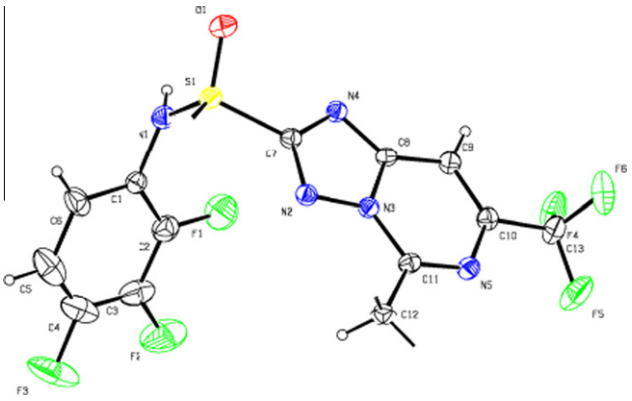


Figure 1. Molecular structure of **8e**.

(N_2–N_5/C_7–C_9) is close to planarity with a maximum deviation of 0.010 Å for C_7. The dihedral angle between the triazolopyrimidine system and the benzene ring (C_1–C_6) is 52.68°.

3.2. Inhibition activity against *At* AHAS

The k_i values against *A. thaliana* AHAS of the synthesized compounds **3a**–**h** and **8a**–**p** were listed in Table 1. FS and Y6610 were used as a positive control. As shown in Table 1, compounds **3d**, **3f**, **8h**, **8j**, and **8m** showed higher enzyme inhibitory activity than Y6610, among which compounds **3d** (k_i = 1.61 μM) and **8h** (k_i = 1.29 μM) exhibited a little higher enzyme inhibitory activity than FS (k_i = 1.85 μM). In addition, structure–activity relationships analysis indicated that the substitution position at benzene ring of compounds **3** and **8** has significant effect on the enzyme inhibition activity. For example, compounds **3a**, **3e**, **3h**, **8a**, **8d**, **8e**, **8f**, and **8l** who bearing substituents at position-4 always showed very low activity. On the contrary, compounds bearing substituents both at position-2 and position-6 always showed higher activity. For example, **3d** (2-Cl-6-COOMe) is the most potent compound within the series of 1,2,4-triazolo[1,5-*a*]-pyrimidine. In order to understand why compound **3d** showed 11.5-folds higher enzyme-inhibiting activity, we carried out molecular simulations by using the same computational protocol as described previously.[14] As shown in Figure 2, the simulated models indicated that compound **3d** binds with *A. thaliana* AHAS in a similar mode to Y6610. Their triazolopyrimidinyl moiety formed π–π stacking interactions with residue Trp574, which made a great contribution to the binding free energies. However, compared with the structure of AHAS-Y6610 complex, compound **3d** formed stronger hydrogen-bonding interactions with the enzyme. For example, Y6610 formed only two hydrogen bonds with residue Arg377 and one hydrogen bond with residue Lys'256, but compound 3d formed two hydrogen bonds with residue Arg377, one hydrogen bond with residue Lys'256, and two additional hydrogen bonds with residues Arg'198 and Ser653 due to the existence of ester group. Therefore, two additional hydrogen bonds should be the accounts for its 11.5-folds higher binding affinity of compound **3d**.

Besides, the series of 1,2,4-triazolo[1,5-*c*]pyrimidine **8a**–**n** showed very similar structure–activity relationships to **3a**–**h**. However, it should be pointed out that bulky and electron-withdrawing substituents at position-2 and position-6 are more favorable for the enzyme inhibition activity. As for the substituent R^1 of compound **8a**–**n**, CH_3 rather than CF_3 seems to be favorable. For example, compound **8g** (R^1 = CH_3, R^2 = H, R = 2-F) showed higher activity than **8i** (R^1 = CF_3, R^2 = H, R = 2-F). Besides, the effects of R^2 substituents on the enzyme inhibition activity seem not very clear. Therefore, more derivatives bearing diverse substituents of R^2 need to be synthesized in the future to understand the structure–activity relationships.

3.3. Greenhouse herbicidal activities

The post-emergence herbicidal activity of series **3** and **8** were tested in greenhouse at the concentration of 150 g.ai/ha, FS was selected as a positive control. As shown in Table 1, all the compounds did not show obvious herbicidal activities against monocotyledon weeds such as *E. crusgalli*, *D. sanguinalis*, and *P. annua*. In addition, seven compounds (**3a**, **3e**, **3h**, **8a**, **8d**, **8e**, and **8f**) with k_i values of over 1000 μM against *At* AHAS did not display obvious herbicidal activities against the tested weeds. Overall, the compounds with higher enzyme inhibition activity showed higher herbicidal activity against dicotyledon weeds such as *B. juncea, A. retroflexus*, and *E. prostrate*. For example, compound **3d** with the highest enzyme inhibition activity within the series of 1,2,4-triazolo[1,5-*a*]-pyrimidine displayed over 90% inhibition activity against *B. juncea, A. retroflexus*, and *E. prostrate* at the dosage of 150 g ai/ha. Compound **8h** with the highest enzyme inhibition activity and compound **8j** with the second-highest enzyme inhibition activity within the series of 1,2,4-triazolo[1,5-*c*]pyrimidine showed good or excellent herbicidal activity against *B. juncea* and *E. prostrate* at the dosage of 150 g ai/ha. Therefore, in order to evaluate the potential of these newly synthesized compounds, six compounds (**3d**, **3f**, **3j**, **8b**, **8h**, **8j**,) having over 80% inhibition activity against at least two kinds of weeds were selected for further test against eight dicotyledon weeds such as *G. aparine, P. humifusum, C. arvense, C. iria*, *C. album*, *A. retroflexus, R. acetasa*, and *E. prostrate*. As shown in Table 2, **3d** seems to be the most promising candidate. Even at

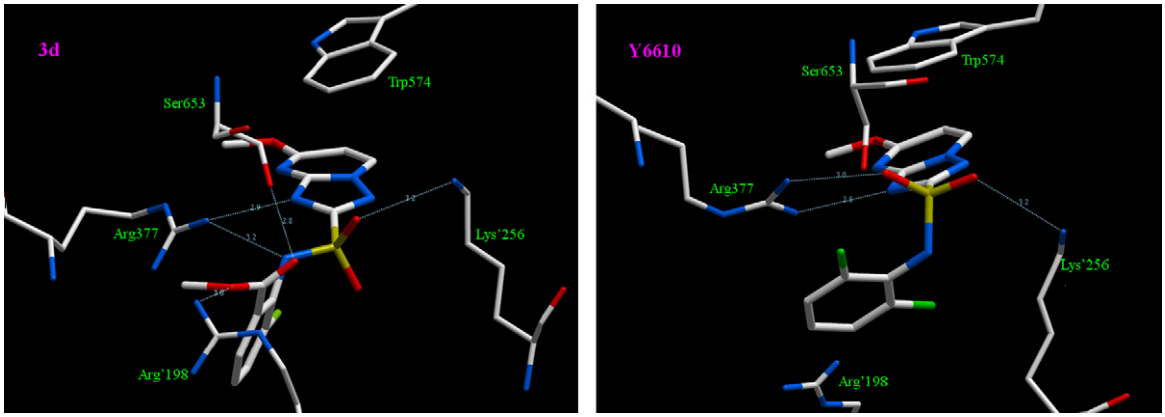


Figure 2. The simulated binding models of **3d** and **Y6610** with *A. thaliana* AHAS.

the concentration of 37.5 g.ai/ha, **3d** showed excellent herbicidal activity with broad spectrum. Its inhibition effects against *G. aparine, C. arvense, C. album*, *A. retroflexus*, and *R. acetasa* are over 80%. At the same time, **3d** also displayed moderate herbicidal activity against *P. humifusum*, *C. iria* and *E. prostrate* at the concentration of 37.5 g.ai/ha. In addition, compound **3d** showed better herbicidal activities against *G. aparine, C. arvense*, and *R. acetasa* than FS at the concentration of 37.5 g.ai/ha. However, FS displayed higher herbicidal activities against *G. aparine, P. humifusum,* and *C. arvense* than *P. humifusum, C. arvense, A. retroflexus*, and *E. prostrate* at the concentration of 37.5 g.ai/ha. As for the weed of *C. iria*, **3d** showed the same level of herbicidal activity as FS.

In summary, a series of 1,2,4-triazolo[1,5-*a*]pyrimidine-2-sulfonamides and 1,2,4-triazolo[1,5-*c*]pyrimidine-2-sulfonamides were designed and synthesized as potential AHAS inhibitors. The results of in vitro and greenhouse test indicated that some compounds showed good AHAS inhibition activity and herbicidal activities at the concentration of 150 g.ai/ha. Most interestingly, **3d**, methyl 3-chloro-2-(5-methoxy-1,2,4-triazolo[1,5-*a*]pyrimidine-2-sulfonamido)benzoate, was identified as the most promising candidate due to a little higher *At* AHAS inhibition effect (k_i = 1.61 μM) than FS and broad spectrum herbicidal activity at the concentration of 37.5 g.ai/ha. The further studies of crop selectivity, field trial and soil residue of **3d** are under the way.

Acknowledgments

The research was supported in part by the National Basic Research Program of China (No. 2010CB126103), the NSFC (Nos. 20925206, 20932005, and 20702018).

References and notes

1. Kleschick, W. A.; Costales, M. J.; Dunbar, J. E.; Meikle, R. W.; Monte, W. T.; Pearson, N. R.; Snider, S. W.; Vinogradoff, A. P. *Pest. Sci.* **1990**, *29*, 341.
2. Whitcomb, C. E. *Toxicol. Ind. Health* **1999**, *15*, 231.
3. Schloss, J. V. *Pest. Sci.* **1990**, *29*, 283.
4. Kleschick, W. A.; Gerwick, B. C.; Carson, C. M.; Monte, W. T.; Snider, S. W. *J. Agric. Food Chem.* **1992**, *40*, 1083.
5. Johnson, T. C.; Martin, T. P.; Mann, R. K.; Pobanz, M. A. *Bioorg. Med. Chem.* **2009**, *17*, 4230.
6. Shaw, D. R.; Bennett, A. C.; Grant, D. L. *Weed Technol.* **1999**, *13*, 791.
7. Butcher, S.; Maycock, R.; Hastings, M.; Watson, P.; Long, T. *Agro Food Ind. Hi Tech.* **1996**, *7*, 36.
8. Krieger, M. S.; Yoder, R. N.; Gibson, A. *J. Agric. Food Chem.* **2000**, *48*, 3710.
9. Yoder, R. N.; Huskin, M. A.; Lennard, L. M.; Zabik, J. M. *J. Agric. Food Chem.* **2000**, *48*, 4335.
10. Gonzalez, M. A.; Gorman, D. B.; Hamilton, C. T.; Roth, G. A. *Org. Process Res. Dev.* **2008**, *12*, 301.
11. Subramanian, M. V.; Loney-Gallant, V.; Dias, J. M.; Mireles, L. C. *Plant Physiol.* **1991**, *96*, 310.
12. Lehmann, R. G.; Fontaine, D. D.; Olberding, E. I. *Weed Res.* **1993**, *33*, 187.
13. Sarfalvi, B.; Nemeth, I.; Szabo, R. *Pflanzenk. Pflanzen.* **2002**, 1059.
14. Chen, C. N.; Lv, L. L.; Ji, F. Q.; Chen, Q.; Xu, H.; Niu, C. W.; Xi, Z.; Yang, G. F. *Bioorg. Med. Chem.* **2009**, *17*, 3011.
15. Boyer, T.; Fournel, L.; Nicolai, E.; Tculon, M. *Heterocycl. Chem.* **1996**, *33*, 1307.
16. John, C. Van H.; Ben, C. G.; William, A. K.; Timothy, C. J. US 5163995, 1992.
17. Sheldrick, G. M. SHELXTL (Version 5.0); University of GÖttingen: GÖttingen, Germany, 2001.
18. *SMART V5.628*, *SAINT V6.45*, and *SADABS*; Bruker AXS Inc.: Madison, WI, 2001.
19. Chang, A. K.; Duggleby, R. G. *Biochem. J.* **1997**, *327*, 161.
20. Xi, Z.; Niu, C. W.; Li, Q. X.; Ouyang, Z.; Ban, S. R. *Chin. J. Pest. Sci.* **2005**, *7*, 215.
21. Li, Y. X.; Luo, Y. P.; Xi, Z.; Niu, C. W.; He, Y. Z.; Yang, G. F. *J. Agric. Food Chem.* **2006**, *54*, 9135.
22. Luo, Y. P.; Jiang, L. L.; Wang, G. D.; Chen, Q.; Yang, G. F. *J. Agric. Food Chem.* **2008**, *56*, 2118.
23. Jiang, L. L.; Tan, Y.; Zhu, X. L.; Wang, Z. F.; Zuo, Y.; Chen, Q.; Xi, Z.; Yang, G. F. *J. Agric. Food Chem.* **2010**, *58*, 2643.