Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 602-608

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Synthesis and Antifungal Evaluation of 1,2,4-Triazolo[1,5-α]pyrimidine Bearing 1,2,4-Triazole Heterocycle Derivatives

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(*Received*: 19 February 2010; *Accepted*: 24 September 2010) AJC-9119

In order to search novel fungicides with higher activity, 28 new 1,2,4-triazolo[1,5-α]pyrimidine derivatives bearing 1,2,4-triazole heterocycle were synthesized. Their structures were characterized by ^{1}H NMR spectroscopy, mass spectrometry and elemental analyses. With triadimefon, validamycin and carbendazim as positive controls, the antifungal activities of 28 compounds against *Fusarium oxysporum* f. sp. *vasinfectum*, *Gibberella sanbinetti*, *Cercospora beticola* Sacc., *Physaclospora piricola* and *Rhizoctonia solani* were evaluated. Compound 2-[(5-(2,6-difluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl[1,2,4]triazolo[1,5-α]pyrimidine (**19**) showed potent antifungal activities against *G. sanbinetti*, *C. beticola*, *P. piricola* and *R. solani*. On the basis of the biological results, structure-activity relationships of these compounds were also discussed.

Key Words: 1,2,4-Triazolo[1,5-α]pyrimidine, 1,2,4-Triazole, Antifungal activity, SAR.

INTRODUCTION

Syntheses and biological activities of novel heterocyclic compounds bearing triazolopyrimidine rings have been studied widely in the fields of medicinal and agricultural chemistry[1-5]. Some triazolopyrimidine derivatives have antifungal activity[2] and can be used as cardiovascular vasodilators[1] and dual thrombin/factor Xa inhibitors[4]. Many triazolopyrimidine-2-sulfonamide derivatives are developed into variety of acetolactate synthase-inhibiting herbicides, such as cloransulam-methyl[5] and metosulam[3]. On the other hand, 1,2,4-triazole derivatives are also typical five-member azole heterocyclic compounds and widely studied by agro-chemists for their high performance, good selectivity and biodegradation rate[6,7]. Cafenstrole is commercially available as carbamoyl triazole herbicide with good selectivity between weed and rice[6]. Many triazoles are fairly good fungicides, which act as ergosterol biosynthesis inhibitors[7].

In our previous studies, we had introduced 1,3,4-oxadiazolyl heterocycle into 1,2,4-triazolo[1,5-α]pyrimidine and gotten novel lead compounds with fairly good antifungal activity[8-10]. In this paper, in order to further study the biological activities of diheterocyclic compounds containing 1,2,4-triazolo[1,5-α]pyrimidine and 1,2,4-triazole and find potent fungicides, we have connected 1,2,4-triazolo[1,5-α]pyrimidine and 1,2,4-triazole with thioether bond by the method of splicing active substructures. Thus, **28** novel 1,2,4-triazolo[1,5-α]pyrimidine containing 1,2,4-triazole heterocycle derivatives were synthesized. Their antifungal activities against *Fusarium oxysporum* f. sp. *vasinfectum* (*F. oxysporum*), *Gibberella sanbinetti* (*G. sanbinetti*), *Cercospora beticola* Sacc. (*C. beticola*), *Physaclospora piricola* (*P. piricola*) and *Rhizoctonia solani* (*R. solani*) were also evaluated with three commercial fungicides, triadimefon, validamycin and carbendazim, used as positive controls.

EXPERIMENTAL

All the reagents were analytical reagent or chemical pure. ^{1}H NMR spectra were recorded at 300 MHz on ^{1}H-Varian-Mercury-300 spectrometers in DMSO-d_6, using TMS as internal standard. Mass spectra were recorded with a Finnigan Trace MS spectrometer. Elementary analyses were performed on a Vario EL III elementary analysis instrument. Melting points were obtained by using an electrothermal digital melting point apparatus. TLC was run on the silica gel coated aluminum sheets (silica gel 60 GF_{254}, E. Merck, Germany) and visualized in UV light (254 nm).

Synthesis of 2-(2-(5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidin-2-ylthio)acetyl)-N-phenylhydrazinecarbothioamide (M_6): M_5 (1.26 g, 5 mmol) and phenyl isothiocyanate (0.80 g, 6 mmol) were dissolved in 60 mL EtOH. The solution was heated and refluxed for 8 h. Then solid was precipitated and filtrated. Recrystallization from DMF/H_2O gave M_6 as white powder, yield: 95.5 %, m.p. 201-203 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.03 (s, 1H, -NH-), 2.35 (s, 3H, 5-CH_3), 2.52 (s, 3H, 7-CH_3), 4.05 (s, 2H, -CH_2-), 7.08 (s, 1H, 6-H), 7.20-7.70 (m, 5H, Ar-H × 5), 10.10 (s, 1H, -NH-), 12.55 (s, -NH-). Anal. (%) ($C_{16}H_{17}N_7OS_2$): C 49.59, H 4.42, N 25.30; found (%): C 49.46, H 4.28, N 25.09.

Synthesis of 5-((5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidin-2-ylthio)methyl)-4-phenyl-4*H*-1, 2,4-triazole-3-thiol (M_7): M_6 (1.94 g, 5 mmol) was dissolved in Na_2CO_3 solution (50 mL, 5 %). Then the solution was heated and refluxed for 4 h. After cooling, crystals were precipitated and filtrated, affording M_7 as colourless crystals, yield: 97.3 %, m.p. 211-213 ºC. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.55 (s, 3H, 5-CH_3), 2.61 (s, 3H, 7-CH_3), 4.41 (s, 2H, -CH_2-), 7.12 (s, 1H, 6-H), 7.42-7.53 (m, 5H, Ar-H × 5), 13.92 (s, 1H, -SH). EI MS: m/z (%) 370.1 ([M + 1]$^+$, 27.1), 369.1 (M$^+$, 86.9), 181.1 (21.7), 180.0 (100.0), 149.0 (48.5), 107.0 (28.2), 76.9 (38.1), 67.0 (23.40). Anal. (%) ($C_{16}H_{15}N_7S_2$): C 52.01, H 4.09, N 26.54; found (%): C 49.87, H 4.09, N 26.37.

General experimental procedure for the synthesis of compound 1-28: M_7 (1.84 g, 5 mmol) and NaOH (0.2 g, 5 mmol) were dissolved in 15 mL water and the solution was stirred to clarifying solution. Then 3 mL MeOH or DMF with 6 mmol different halohydrocarbon dissolved in was added carefully and the solution was stirred at room temperature for 9-56 h. Solid was precipitated and filtrated. Recrystallization from different organic solvents gave target compounds with different physical states.

5,7-Dimethyl-2-[(5-(2-methylbenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-[1,2,4]triazolo[1,5-α]pyrimidine (1): White crystals (methanol), reaction time 24 h, m.p. 168-169 ºC, yield 51.3 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.23 (s, 2H, 2-CH_3Ph), 2.58 (s, 3H, 5-CH_3), 2.63 (s, 3H, 7-CH_3), 4.38 (s, 2H, -CH_2Ph), 4.56 (s, 2H, -SCH_2-), 6.69 (s, 1H, 6-H), 7.02-7.30 (m, 9H, Ar-H × 9). EI MS: m/z (%) 473 (M$^+$, 16.3), 293 (8.2), 261 (1.7), 221 (8.8), 193 (4.1), 181 (14.6), 180 (21.5), 107 (13.8), 105 (100), 77 (26.5). Anal. (%) ($C_{24}H_{23}N_7S_2$): C 60.86, H 4.89, N 20.70; found (%): C 60.72, H 4.89, N 20.53.

5,7-Dimethyl-2-[(5-(3-methylbenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-[1,2,4]triazolo[1,5-α]pyrimidine (2): White flocculent crystals (methanol), reaction time 25 h, m.p. 136-138 ºC, yield 65.7 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.27 (s, 2H, 3-CH_3Ph), 2.61 (s, 3H, 5-CH_3), 2.63 (s, 3H, 7-CH_3), 4.35 (s, 2H, -CH_2Ph), 4.58 (s, 2H, -SCH_2-), 6.71 (s, 1H, 6-H), 7.03-7.33 (m, 9H, Ar-H × 9). EI MS: m/z (%) 474 ([M + 1]$^+$, 41.3), 473 (M$^+$, 72.9), 293 (33.6), 261 (4.1), 221 (51.3), 193 (16.1), 181 (82.4), 180 (81.5), 108 (31.1), 107 (45.05), 105 (100), 77 (88.2). Anal. (%) ($C_{24}H_{23}N_7S_2$): C 60.86, H 4.89, N 20.70; found (%): C 60.71, H 4.82, N 20.63.

5,7-Dimethyl-2-[(5-(4-methylbenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-[1,2,4]triazolo[1,5-α]pyrimidine (3): Yellow flocculent crystals (dichloromethane/petroleum ether), reaction time 23 h, m.p. 144-146 ºC, yield 75.4 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.26 (s, 2H, 4-CH_3Ph), 2.56 (s, 3H, 5-CH_3), 2.61 (s, 3H, 7-CH_3), 4.33 (s, 2H, -CH_2Ph), 4.55 (s, 2H, -SCH_2-), 6.68 (s, 1H, 6-H), 7.01-7.22 (m, 9H, Ar-H × 9). EI MS: m/z (%) 474 ([M + 1]$^+$, 17.8), 473 (M$^+$, 41.0), 293 (25.2), 261 (2.28), 221 (40.1), 193 (8.6), 181 (57.3), 180 (56.7), 108 (19.2), 107 (29.2), 105 (100), 77 (57.1). Anal. (%) ($C_{24}H_{23}N_7S_2$): C 60.86, H 4.89, N 20.70; found (%): C 61.19, H 4.58, N 20.84.

2-[(5-(2-Chlorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (4): Yellow flocculent crystals (dichloromethane/petroleum ether), reaction time 22 h, m.p. 155-157 ºC, yield 81.8 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.59 (s, 3H, 5-CH_3), 2.64 (s, 3H, 7-CH_3), 4.48 (s, 2H, -CH_2Ph), 4.56 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 7.12-7.43 (m, 9H, Ar-H × 9). EI MS: m/z (%) 495 ([M + 2]$^+$, 6.1), 493 (M$^+$, 13.1), 458 (35.0), 279 (10.7), 237 (13.8), 181 (9.7), 180 (18.4), 127 (32.33), 125 (100), 108 (13.3), 107 (18.2), 89 (19.2), 77 (20.8). Anal. (%) ($C_{23}H_{20}N_7S_2Cl$): C 55.92, H 4.08, N 19.85; found (%): C 55. 65, H 4.17, N 19.62.

2-[(5-(3-Chlorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (5): With needles (methanol), reaction time 24 h, m.p. 170-173 ºC, yield 64.4 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.58 (s, 3H, 5-CH_3), 2.61 (s, 3H, 7-CH_3), 4.31 (s, 2H, -CH_2Ph), 4.54 (s, 2H, -SCH_2-), 6.69 (s, 1H, 6-H), 7.11-7.36 (m, 9H, Ar-H × 9). EI MS: m/z (%) 495 ([M + 2]$^+$, 8.4), 493 (M$^+$, 18.1), 356 (13.0), 297 (4.6), 237 (12.1), 181 (15.3), 180 (23.3), 127 (31.8), 125 (100), 108 (16.3), 107 (20.8), 89 (21.0), 77 (22.4). Anal. (%) ($C_{23}H_{20}N_7S_2Cl$): C 55.92, H 4.08, N 19.85; found (%): C 55.85, H 3.92, N 19.73.

2-[(5-(4-Chlorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (6): With solids (dichloromethane/petroleum ether), reaction time 23 h, m.p. 162-164 ºC, yield 90.3 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.62 (s, 3H, 5-CH_3), 2.67 (s, 3H, 7-CH_3), 4.37 (s, 2H, -CH_2Ph), 4.59 (s, 2H, -SCH_2-), 6.73 (s, 1H, 6-H), 7.18-7.35 (m, 9H, Ar-H × 9). EI MS: m/z (%) 495 ([M + 2]$^+$, 1.9), 493 (M$^+$, 4.0), 237 (5.9), 181 (7.0), 180 (10.0), 127 (33.4), 125 (100), 108 (12.4), 107 (15.1), 89 (15.4), 77 (19.8). Anal. (%) ($C_{23}H_{20}N_7S_2Cl$): C 55.92, H 4.08, N 19.85; found (%): C 55.55, H 3.82, N 20.05.

2-[(5-(2-Bromobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]-pyrimidine (7): White flocculent crystals (methanol), reaction time 21 h, m.p. 139-141 ºC, yield 62.7 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.57 (s, 3H, 5-CH_3), 2.62 (s, 3H, 7-CH_3), 4.49 (s, 2H, -CH_2Ph), 4.55 (s, 2H, -SCH_2-), 6.69 (s, 1H, 6-H), 7.05-7.48 (m, 9H, Ar-H × 9). EI MS: m/z (%) 539 ([M + 2]$^+$, 8.9), 537 (M$^+$, 17.6), 458 (81.3), 279 (28.5), 237 (35.9), 181 (21.9), 180 (46.7), 171 (99.4), 169 (100), 149 (25.2), 108 (30.3), 107 (40.8), 89 (41.7), 77 (49.9). Anal. (%) ($C_{23}H_{20}N_7S_2Br$): C 51.30, H 3.74, N 18.21; found (%): C 51.37, H 3.83, N 18.46.

2-[(5-(3-Bromobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]-pyrimidine (8): White crystals (methanol), reaction time 23 h,

m.p. 187-190 ºC, yield 75.3 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.59 (s, 3H, 5-CH_3), 2.64 (s, 3H, 7-CH_3), 4.32 (s, 2H, -CH_2Ph), 4.57 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 7.14-7.41 (m, 9H, Ar-H × 9). EI MS: m/z (%) 539 ([M + 2]$^+$, 46.8), 537 (M$^+$, 45.7), 458 (9.6), 279 (22.8), 237 (50.3), 181 (53.6), 180 (75.3), 171 (99.0), 169 (100), 149 (47.2), 108 (39.4), 107 (50.6), 89 (56.2), 77 (73.4). Anal. (%) ($C_{23}H_{20}N_7S_2Br$): C 51.30, H 3.74, N 18.21; found (%): C 51.48, H 3.52, N 18.03.

2-[(5-(4-Bromobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (9): White solids (dichloromethane/petroleum ether), reaction time 20 h, m.p. 156-157 ºC, yield 79.5 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.61 (s, 3H, 5-CH_3), 2.67 (s, 3H, 7-CH_3), 4.34 (s, 2H, -CH_2Ph), 4.59 (s, 2H, -SCH_2-), 6.73 (s, 1H, 6-H), 7.14-7.48 (m, 9H, Ar-H × 9). EI MS: m/z (%) 539 ([M + 2]$^+$, 60.4), 537 (M$^+$, 62.6), 458 (4.8), 279 (3.8), 237 (75.5), 181 (73.7), 180 (82.3), 171 (93.4), 169 (100), 149 (75.5), 108(60.0), 107 (83.1), 89 (81.2), 77 (95.7). Anal. (%) ($C_{23}H_{20}N_7S_2Br$): C 51.30, H 3.74, N 18.21; found (%): C 51.13, H 3.57, N 18.12.

2-[(5-(2-Fluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (10): Pale yellow solids (dichloromethane/petroleum ether), reaction time 23 h, m.p. 96-98 ºC, yield 80.8 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.59 (s, 3H, 5-CH_3), 2.65 (s, 3H, 7-CH_3), 4.42 (s, 2H, -CH_2Ph), 4.58 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 7.00-7.37 (m, 9H, Ar-H × 9). EI MS: m/z (%) 478 ([M + 1]$^+$, 7.9), 477 (M$^+$, 23.2), 340 (18.3), 237 (10.8), 181 (9.5), 180 (13.7), 149 (11.1), 109 (100), 107 (18.9), 89 (4.6), 77 (17.5). Anal. (%) ($C_{23}H_{20}N_7S_2F$): C 57.84, H 4.22, N 20.53; found (%): C 57.96, H 4.03, N 20.34.

2-[(5-(3-Fluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (11): White needles (methanol), reaction time 25 h, m.p. 88-91 ºC, yield 49.4 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.62 (s, 3H, 5-CH_3), 2.67 (s, 3H, 7-CH_3), 4.39 (s, 2H, -CH_2Ph), 4.60 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 7.00-7.34 (m, 9H, Ar-H × 9). EI MS: m/z (%) 478 ([M + 1]$^+$, 3.9), 477 (M$^+$, 57.2), 368 (37.7), 340 (51.7), 237 (50.0), 220 (44.8), 193 (22.1), 181 (44.7), 180 (47.6), 149 (45.0), 109(100), 107 (52.3), 89 (16.0), 77 (55.4). Anal. (%) ($C_{23}H_{20}N_7S_2F$): C 57.84, H 4.22, N 20.53; found (%): C 58.06, H 3.90, N 20.17.

2-[(5-(4-Fluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (12): White crystals (methanol), reaction time 223 h, m.p. 146-147 ºC, yield 54.4 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.58 (s, 3H, 5-CH_3), 2.63 (s, 3H, 7-CH_3), 4.34 (s, 2H, -CH_2Ph), 4.56 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 6.92-7.33 (m, 9H, Ar-H × 9). EI MS: m/z (%) 478 ([M + 1]$^+$, 33.6), 477 (M$^+$, 52.4), 340 (37.5), 237 (35.6), 181 (49.0), 180 (53.7), 149 (44.8), 109 (100), 107 (51.8), 89 (13.6), 77 (57.4). Anal. (%) ($C_{23}H_{20}N_7S_2F$): C 57.84, H 4.22, N 20.53; found (%): C 58.06, H 4.34, N 20.67.

5,7-Dimethyl-2-[(5-(3-nitrobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-[1,2,4]triazolo[1,5-α]pyrimidine (13): Pale yellow crystals (dichloromethane/petroleum ether), reaction time 24 h, m.p. 178-179 ºC, yield 90.3 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.60 (s, 3H, 5-CH_3), 2.66 (s, 3H, 7-CH_3), 4.47 (s, 2H, -CH_2Ph), 4.57 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 7.18-7.44 (m, 9H, Ar-H × 9). EI MS: m/z (%) 505 ([M + 1]$^+$, 14.5), 504 (M$^+$, 38.9), 367 (48.0), 279 (2.5), 237 (31.0), 181 (21.4), 180 (43.2), 149 (32.5), 136 (100), 108(26.9), 107 (37.0), 89 (57.5), 77 (45.5). Anal. (%) ($C_{23}H_{20}N_8O_2S_2$): C 54.75, H 4.00, N 22.21; found (%): C 54.32, H 4.19, N 22.17.

2-[(5-(3-Methoxybenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidine (14): White crystals (methanol), reaction time 19 h, m.p. 139-141 ºC, yield 73.0 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.55 (s, 3H, 5-CH_3), 2.60 (s, 3H, 7-CH_3), 3.67 (s, 3H, 4-OCH_3Ph), 4.31 (s, 2H, -CH_2Ph), 4.54 (s, 2H, -SCH_2-), 6.67 (s, 1H, 6-H), 7.10-7.29 (m, 9H, Ar-H × 9). EI MS: m/z (%) 490 ([M + 1]$^+$, 22.3), 489 (M$^+$, 52.5), 369 (16.2), 279 (1.5), 237 (39.7), 193 (12.8), 181 (45.9), 180 (71.2), 149 (31.7), 121(100), 108 (26.0), 107 (37.8), 89 (10.3), 77 (70.7). Anal. (%) ($C_{24}H_{23}N_7OS_2$): C 58.87, H 4.37, N 20.03; found (%): C 58.65, H 4.29, N 19.93.

5,7-Dimethyl-2-[(4-phenyl-5-(4-vinylbenzylthio)-4*H*-1,2,4-triazol-3-yl)methylthio]-[1,2,4]triazolo[1,5-α]pyrimidine (15): White solids (dichloromethane/petroleum ether), reaction time 25 h, m.p. 136-138 ºC, yield 92.1 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.57 (s, 3H, 5-CH_3), 2.62 (s, 3H, 7-CH_3), 4.31 (s, 2H, -CH_2Ph), 4.55 (s, 2H, -SCH_2-), 5.20 (q, *J* = 7.5 Hz, 1H, -CH=), 5.70 (d, *J* = 7.5 Hz, 2H, -CH_2=), 6.57 (s, 1H, 6-H), 7.11-7.28 (m, 9H, Ar-H × 9). EI MS: m/z (%) 485 (M$^+$, 0.7), 369 (0.9), 237 (1.1), 193 (22.2), 181 (22.3), 180 (6.9), 149 (5.0), 117 (100), 115 (44.8), 108 (5.3), 107 (6.9), 91 (23.4), 77 (18.4). Anal. (%) ($C_{25}H_{23}N_7S_2$): C 61.83, H 4.77, N 20.19; found (%): C 62.00, H 4.52, N 20.36.

5,7-Dimethyl-2-[(5-(4-methyl-2-nitrobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-[1,2,4]triazolo-[1,5-α]pyrimidine (16): Yellow crystals (dichloromethane/petroleum ether), reaction time 24 h, m.p. 177-179 ºC, yield 88.4 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.37 (s, 3H, 4-CH_2Ph), 2.58 (s, 3H, 5-CH_3), 2.62 (s, 3H, 7-CH_3), 4.55 (s, 2H, -CH_2Ph), 4.72 (s, 2H, -SCH_2-), 6.69 (s, 1H, 6-H), 7.12-7.55 (m, 8H, Ar-H × 8). EI MS: m/z (%) 519 ([M + 1]$^+$, 1.0), 518 (M$^+$, 2.9), 369 (5.4), 279 (1.1), 237 (9.0), 180 (31.8), 149 (26.6), 134 (40.1), 108 (20.8), 107 (33.6), 89 (10.2), 77 (100). Anal. (%) ($C_{24}H_{22}N_8O_2S_2$): C 55.58, H 4.28, N 21.61; found (%): C 55.62, H 4.39, N 21.73.

5,7-Dimethyl-2-[(5-(4-methyl-3-nitrobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl) methylthio]-[1,2,4]triazolo-[1,5-α]pyrimidine (17): Yellow crystals (dichloromethane/petroleum ether), reaction time 25 h, m.p. 199-200 ºC, yield 75.0 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.55 (s, 3H, 4-CH_2Ph), 2.60 (s, 3H, 5-CH_3), 2.65 (s, 3H, 7-CH_3), 4.40 (s, 2H, -CH_2Ph), 4.57 (s, 2H, -SCH_2-), 6.71 (s, 1H, 6-H), 7.18-7.53 (m, 8H, Ar-H × 8). EI MS: m/z (%) 519 ([M + 1]$^+$, 19.3), 518 (M$^+$, 56.0), 369 (26.4), 368 (14.6), 237 (32.5), 232 (23.8), 181 (36.4), 180 (75.4), 150 (100), 149 (46.2), 134 (54.8), 108 (35.2), 107 (49.7), 89 (10.4), 77 (85.7). Anal. (%) ($C_{24}H_{22}N_8O_2S_2$): C 55.58, H 4.28, N 21.61; found (%): C 55.37, H 4.32, N 21.53.

2-[(5-(3,4-Difluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (18): Pale yellow crystals (methanol), reaction time 15 h, m.p. 144-146 ºC, yield 72.3 %. ^{1}H NMR (300 MHz,

DMSO-d_6): δ 2.59 (s, 3H, 5-CH_3), 2.64 (s, 3H, 7-CH_3), 4.33 (s, 2H, -CH_2Ph), 4.57 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 7.00-7.38 (m, 8H, Ar-H × 8). EI MS: m/z (%) 496 ([M + 1]$^+$, 3.5), 495 (M$^+$, 11.2), 369 (2.3), 358 (8.2), 237 (7.6), 181 (6.2), 180 (8.1), 149 (9.3), 134 (7.9), 127 (100), 108 (10.9), 107 (17.2), 89 (1.5), 77 (19.3). Anal. (%) ($C_{23}H_{19}N_7S_2F_2$): C 55.74, H 3.86, N 19.78; found (%): C 55.51, H 3.97, N 19.66.

2-[(5-(2,6-Difluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (19): White flocculent crystals (methanol), reaction time 19 h, m.p. 101-103 ºC, yield 51.6 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.62 (s, 3H, 5-CH_3), 2.68 (s, 3H, 7-CH_3), 4.42 (s, 2H, -CH_2Ph), 4.63 (s, 2H, -SCH_2-), 6.80 (s, 1H, 6-H), 7.20-7.38 (m, 8H, Ar-H × 8). EI MS: m/z (%) 495 (M$^+$, 2.9), 237 (3.0), 181 (1.5), 180 (3.1), 149 (4.7), 134 (5.4), 127 (100), 108 (8.1), 107 (14.3), 89 (1.2), 77 (17.6). Anal. (%) ($C_{23}H_{19}N_7S_2F_2$): C 55.74, H 3.86, N 19.78; found (%): C 55.63, H 4.02, N 19.64.

2-[(5-(2,4-Difluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (20): White crystals (methanol), reaction time 17 h, m.p. 128-130 ºC, yield 57.1 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.58 (s, 3H, 5-CH_3), 2.63 (s, 3H, 7-CH_3), 4.37 (s, 2H, -CH_2Ph), 4.56 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 7.17-7.43 (m, 8H, Ar-H × 8). EI MS: m/z (%) 496 ([M + 1]$^+$, 5.7), 495 (M$^+$, 17.2), 237 (8.2), 181 (7.6), 180 (13.0), 149 (11.6), 134 (9.7), 127 (100), 108 (12.7), 107 (21.4), 89 (2.1), 77 (16.8). Anal. (%) ($C_{23}H_{19}N_7S_2F_2$): C 55.74, H 3.86, N 19.78; found (%): C 55.86, H 3.75, N 19.92.

2-[(5-(4-Bromo-2-fluorobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo-[1,5-α]pyrimidine (21): White crystals (methanol), reaction time 12 h, m.p. 102-105 ºC, yield 71.9 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.59 (s, 3H, 5-CH_3), 2.65 (s, 3H, 7-CH_3), 4.35 (s, 2H, -CH_2Ph), 4.56 (s, 2H, -SCH_2-), 6.71 (s, 1H, 6-H), 7.14-7.37 (m, 8H, Ar-H × 8). EI MS: m/z (%) = 557 ([M + 2]$^+$, 22.1), 555 (M$^+$, 20.3), 369 (18.0), 237 (26.9), 189 (100), 187 (97.1), 181 (25.4), 180 (56.0), 149 (35.1), 134 (38.0), 127 (2.2), 108 (72.7), 107 (75.6), 89 (6.7), 77 (42.3). Anal. (%) ($C_{23}H_{19}N_7S_2BrF$): C 49.64, H 3.44, N 17.62; found (%): C 49.87, H 3.79, N 17.57.

2-[(5-(Benzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)-methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (22): White crystals (ethanol), reaction time 49 h, m.p. 151-152 ºC, yield 47.3 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.57 (s, 3H, 5-CH_3), 2.63 (s, 3H, 7-CH_3), 4.35 (s, 2H, -CH_2Ph), 4.56 (s, 2H, -SCH_2-), 6.69 (s, 1H, 6-H), 7.01-7.34 (m, 10H, Ar-H × 10). EI MS: m/z (%) 459 (M$^+$, 11.2), 460 (9.3), 237 (15.0), 221 (1.8), 207 (14.9), 193 (5.3), 181 (13.5), 180 (19.7), 108 (15.2), 107 (19.0), 91 (100), 77 (20.3). Anal. (%) ($C_{23}H_{21}N_7S_2$): C 60.11, H 4.61, N 21.33; found (%): C 60.34, H 4.25, N 21.30.

5,7-Dimethyl-2-[(5-(4-nitrobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-[1,2,4]triazolo[1,5-α]pyrimidine (23): White crystals (ethanol), reaction time 20 h, m.p. 164-166 ºC, yield 93.0 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.62 (s, 3H, 5-CH_3), 2.67 (s, 3H, 7-CH_3), 4.48 (s, 2H, -CH_2Ph), 4.57 (s, 2H, -SCH_2-), 6.74 (s, 1H, 6-H), 7.21-7.56 (m, 9H, Ar-H × 9). EI MS: m/z (%) 505 ([M + 1]$^+$, 16.7), 504 (M$^+$, 42.5), 367 (42.3), 279 (4.4), 237 (37.0), 181 (32.5), 180 (100), 149 (50.9), 136 (37.8), 108 (39.3), 107 (62.9), 89 (34.7), 77 (64.4). Anal. (%) ($C_{23}H_{20}N_8O_2S_2$): C 54.75, H 4.00, N 22.21; found (%): C 55.01, H 4.09, N 22.12.

5,7-Dimethyl-2-[(4-phenyl-5-(propylthio)-4*H*-1,2,4-triazol-3-yl)methylthio]-[1,2,4]triazolo[1,5-α]pyrimidine (24): Gray solids (acetone), reaction time 10 h, m.p. 144-145 ºC, yield 63.4 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.98 (t, J = 6.0 Hz, 3H, -CH_3), 1.77 (m, 2H, -CH_2-), 2.62 (s, 3H, 5-CH_3), 2.68 (s, 3H, 7-CH_3), 3.19 (t, J = 6.0 Hz, 2H, -SCH_2-), 4.62 (s, 2H, -SCH_2-), 6.74 (s, 1H, 6-H), 7.30-7.41 (m, 5H, Ar-H × 5). EI MS: m/z (%) 412 ([M + 1]$^+$, 2.0), 411 (M$^+$, 6.9), 232 (29.9), 190 (21.4), 189 (11.6), 181 (4.4), 180 (22.5), 149 (24.6), 136 (18.8), 108 (22.9), 107 (32.9), 77 (78.5). Anal. (%) ($C_{19}H_{21}N_7S_2$): C 55.45, H 5.14, N 23.82; found (%): C 55.41, H 5.09, N 24.02.

2-[(5-(Butylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)-methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (25): White solids (acetone), reaction time 27 h, m.p. 138-139 ºC, yield 48.6 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, J = 6.0 Hz, 3H, -CH_3), 1.33-1.69 (m, 4H, -CH_2-× 2), 2.58 (s, 3H, 5-CH_3), 2.64 (s, 3H, 7-CH_3), 3.17 (t, J = 6.0 Hz, 2H, -SCH_2-), 4.59 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 7.26-7.41 (m, 5H, Ar-H × 5). EI MS: m/z (%) 426 ([M + 1]$^+$, 1.2), 425 (M$^+$, 3.7), 232 (6.3), 190 (9.4), 189 (10.6), 181 (4.6), 180 (20.8), 149 (14.9), 136 (17.2), 108 (7.7), 107 (14.9), 77 (53.8). Anal. (%) ($C_{20}H_{23}N_7S_2$): C 56.44, H 5.45, N 23.04; found (%): C 56.25, H 5.35, N 23.42.

2-[(5-(Ethylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)-methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (26): White solids (acetone), reaction time 9 h, m.p. 161-162 ºC, yield 75.3 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.38 (t, J = 5.5 Hz, 3H, -CH_3), 2.62 (s, 3H, 5-CH_3), 2.67 (s, 3H, 7-CH_3), 3.23 (q, J = 5.5 Hz, 2H, -SCH_2-), 4.62 (s, 2H, -SCH_2-), 6.73 (s, 1H, 6-H), 7.31-7.44 (m, 5H, Ar-H × 5). EI MS: m/z (%) 398 ([M + 1]$^+$, 1.3), 397 (M$^+$, 8.4), 232 (3.2), 190 (18.0), 189 (10.5), 181 (5.7), 180 (29.3), 149 (16.8), 136 (19.4), 108 (11.2), 107 (22.6), 77 (100). Anal. (%) ($C_{18}H_{19}N_7S_2$): C 54.39, H 4.82, N 24.66; found (%): C 54.12, H 4.79, N 24.46.

2-[(5-(Hexylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)-methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (27): Grey solids (acetone), reaction time 56 h, m.p. 126-128 ºC, yield 46.4 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, J = 6.0 Hz, 3H, -CH_3), 1.23-1.72 (m, 8H, -CH_2-× 4), 2.59 (s, 3H, 5-CH_3), 2.65 (s, 3H, 7-CH_3), 3.14 (t, J = 6.0 Hz, 2H, -SCH_2-), 4.55 (s, 2H, -SCH_2-), 6.67 (s, 1H, 6-H), 7.16-7.30 (m, 5H, Ar-H × 5). EI MS: m/z (%) 454 ([M + 1]$^+$, 68.4), 453 (M$^+$, 89.4), 406 (38.0), 369 (43.5), 232 (100), 190 (81.4), 188 (31.3), 181 (64.9), 180 (56.5), 149 (73.0), 148 (62.4), 108 (45.3), 107 (68.8), 77 (91). Anal. (%) ($C_{22}H_{27}N_7S_2$): C 58.25, H 6.00, N 21.61; found (%): C 58.55, H 5.46, N 21.51.

2-((5-(3-Bromopropylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)methylthio]-5,7-dimethyl-[1,2,4]triazolo[1,5-α]pyrimidine (28): White solids (acetone), reaction time 27 h, m.p. 214-216 ºC, yield 77.0 %. ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.98 (t, J = 5.8 Hz, 2H, -CH_2-), 2.18 (m, 2H, -CH_2-), 2.59 (s, 3H, 5-CH_3), 2.65 (s, 3H, 7-CH_3), 3.24 (t, J = 5.8 Hz, 2H, -SCH_2-), 4.57 (s, 2H, -SCH_2-), 6.70 (s, 1H, 6-H), 7.26-7.39 (m, 5H, Ar-H × 5). EI MS: m/z (%) 490 (M$^+$, 0.33), 369 (3.4),

232 (2.5), 190 (6.9), 189 (10.3), 181 (7.9), 180 (35.1), 149 (19.6), 148 (8.2), 108 (9.6), 107 (19.6), 77 (52.1), 67 (37.7), 59 (34.0), 39 (100). Anal. (%) ($C_{19}H_{20}N_7S_2Br$): C 46.53, H 4.11, N 19.99; found (%): C 46.80, H 4.08, N 19.65.

Fungi strains and positive controls: *F. oxysporum*, *G. sanbinetti*, *C. beticola*, *P. piricola* and *R. solani* were provided by the courtesy of the Center for Bioassay, Central China Normal University. Three commercial fungicides, triadimefon, validamycin and carbendazim, were selected as positive controls.

Assay of antifungal activity: Proper samples were weighed out and dissolved in a spot of DMF (half the burette). A blob of emulsion was added and the solution was diluted to 1000 ppm. Potatoes were husked and washed. Then potatoes (200 g) were weighed out, cut up and added into distilled water (700 mL). The water boiled for long time and filtrated by gauze. Sucrose (20 g) and agar (15 g) were added into filtrate and the filtrate was heated to dissolve agar. Then the filtrate was diluted to 1000 mL and divided into two conical flasks. After sterilizing at 128 ºC for 15 min, 10 mL filtrate was added into each culture dish and 9 mL sterilized distilled water and 1 mL 1000 ppm tested sample were added meanwhile. Pure culture fungi were inoculated into the coagulated mediums and the mediums were placed in bacteriological incubator. After cultivation at 25 ºC for more than 48 h, the diffusions of strains were investigated and the diameters of strains were measured. Comparing with the control, the inhibition rates were calculated and the efficacies were evaluated. The inhibition rate was calculated as follows:

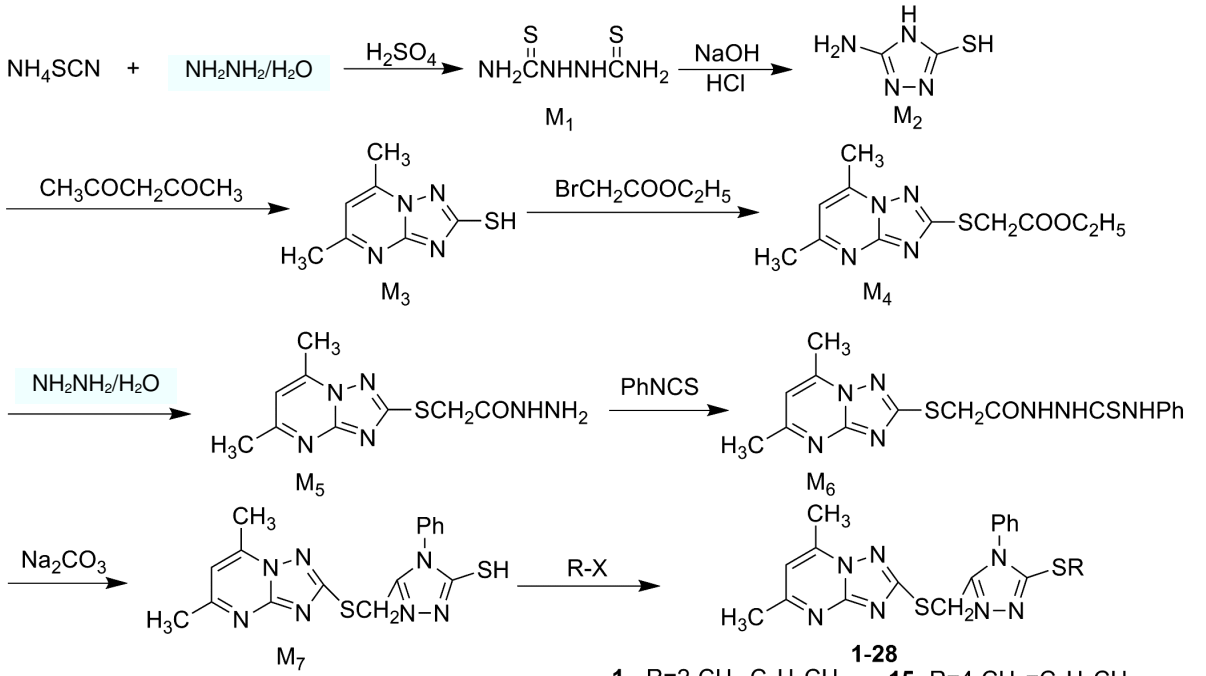
$$\text{Inhibition rate} = \frac{(D_t - D_c)}{D_c} \times 100\ \%$$

where D_t and D_c are the diameter of treated strain and control strain, respectively.

Grades of efficacy: The grades of efficacies were classified as: grade A (inhibition rate = 90 %), grade B (inhibition rate = 70-89 %), grade C (inhibition rate = 50-70 %) and grade D (inhibition rate < 50 %).

RESULTS AND DISCUSSION

Synthesis of intermediates: Begin with hydrazine hydrate, $\mathbf{M_5}$ was synthesized *via* five steps using the reported method (**Scheme-I**)[11]. After being refluxed for 5 h, $\mathbf{M_5}$ and phenyl isothiocyanate afforded a new compound $\mathbf{M_6}$. In the alkali medium, $\mathbf{M_6}$ was cyclized to form $\mathbf{M_7}$. The possible



1 R=2-CH_3-$C_6H_4CH_2$ **15** R=4-CH_2=$C_6H_4CH_2$
2 R=3-CH_3-$C_6H_4CH_2$ **16** R=2-NO_2-4-CH_3-$C_6H_4CH_2$
3 R=4-CH_3-$C_6H_4CH_2$ **17** R=3-NO_2-4-CH_3-$C_6H_4CH_2$
4 R=2-Cl-$C_6H_4CH_2$ **18** R=3,4-F_2-$C_6H_4CH_2$
5 R=3-Cl-$C_6H_4CH_2$ **19** R=2,6-F_2-$C_6H_4CH_2$
6 R=4-Cl-$C_6H_4CH_2$ **20** R=2,4-F_2-$C_6H_4CH_2$
7 R=2-Br-$C_6H_4CH_2$ **21** R=2-F-4-Br-$C_6H_4CH_2$
8 R=3-Br-$C_6H_4CH_2$ **22** R=$C_6H_4CH_2$
9 R=4-Br-$C_6H_4CH_2$ **23** R=4-NO_2-$C_6H_4CH_2$
10 R=2-F-$C_6H_4CH_2$ **24** R=$CH_3CH_2CH_2$
11 R=3-F-$C_6H_4CH_2$ **25** R=$CH_3(CH_2)_2CH_2$
12 R=4-F-$C_6H_4CH_2$ **26** CH_3CH_2
13 R=3-NO_2-$C_6H_4CH_2$ **27** $CH_3(CH_2)_4CH_2$
14 R=3-OCH_3-$C_6H_4CH_2$ **28** $BrCH_2CH_2CH_2$

Scheme-I: Synthesis of **1-28**

reaction mechanism was first enolization of thiocarbonyl, then nucleophilic addition and cyclization with carbonyl at position[1].

Synthesis of target compounds: Target compounds **1-28** were synthesized by S-alkylation of $\mathbf{M_7}$ (**Scheme-I**). During the reaction process, $\mathbf{M_7}$ was dissolved in very dilute sodium hydroxide solution, to afford sulfur anion and attack carbon atom of alkyl halides with partial positive charge. Thus, a typical nucleophilic substitution reaction was occurred. Because halohydrocarbons did not dissolve in water, a spot of organic solvent, which dissolved in water very well, were used to dissolve halohydrocarbons, such as methanol, ethanol and N,N-dimethylformamide. Then dissolved halohydrocarbons were added to the reaction system. Target compounds did not dissolve in water and there was only a spot of organic solvent in the reaction system. So the products would precipitate during the reaction process. It was beneficial to reaction and the precipitated products were very pure. In addition, the formation rates of substituted benzyl sulfides depended on the properties and positions of substituents at benzene ring. Electron-withdrawing groups and *para*-groups were advantageous to such reactions.

Biological activity: With triadimefon, validamycin and carbendazim as positive controls, the antifungal activity of **1-28** against *F. oxysporum*, *G. sanbinetti*, *C. beticola*, *P. piricola* and *R. solani* was evaluated by previous method of the contained poison in the medium[12]. The results summarized in Table-1 show that the inhibitory effects of these compounds on *F. oxysporum* and *G. sanbinetti* were weak and only **14** and **19** reached grade B of the efficacy. However, **11**, **13**, **17-19** had good inhibitory effects on *C. beticola*. Most compounds have very good inhibitory effects on *P. piricola* and *R. solani*. Efficacies of **4, 6, 10, 12, 16-21** and **23-28** reached grade B. On the other hand, many of the compounds had good inhibitory effects on more than two kinds of fungi. **16, 17, 20** and **21** had inhibitory effects on two fungi. **18** had inhibitory effects on three fungi. Furthermore, **19** had inhibitory effects on four fungi. The inhibitory effects of these compounds, of which efficacies reached grade B, were higher than or comparable with positive controls.

As reported, 1,2,4-triazolo [1,5-α]pyrimidine derivatives have many biological activities[1-5]. The trizolopyrimidine structure including the S atom, which is a large delocalized system with rich electron, might play an important role during the process. The biological activities possibly relate with the spatial orientation and electron supplying capacity of the trizolopyrimidine[13]. On the other hand, triazole fungicides are ergosterol biosynthesis inhibitors (EBI) and they have very strong biological activity. The triazole structure is the active group. It can coordinate with porphyrin ring and prevent the formation of iron porphyrin oxygen complex. Thus it strongly inhibits the biosynthesis of ergosterol, which is the important part of cell membrane, so as to antifungal objective.

1-28 have the two active groups and exhibit good antifungal activity. But the antifungal activity of these compounds is quite different. It is possible related to the influence of different substituent R, which has different electrostatic and steric property. Table-1 shows that most of the compounds

TABLE-1
ANTIFUNGAL ACTIVITY OF COMPOUNDS 1-28

Comp.	Inhibition rate (%) (concentration: 50 ppm)				
	F. oxysporum	*G. sanbinetti*	*C. beticola*	*P. piricola*	*R. solani*
1	60 ± 2	38 ± 1	35 ± 4	56 ± 3	54 ± 1
2	55 ± 1	55 ± 1	42 ± 1	31 ± 3	53 ± 3
3	65 ± 2	22 ± 2	37 ± 1	66 ± 3	56 ± 1
4	50 ± 1	47 ± 1	42 ± 1	63 ± 1	**70 ± 3**
5	55 ± 3	16 ± 3	54 ± 3	66 ± 3	52 ± 1
6	40 ± 1	44 ± 1	44 ± 1	66 ± 5	**72 ± 1**
7	58 ± 1	47 ± 1	37 ± 1	68 ± 3	68 ± 1
8	62 ± 3	25 ± 1	42 ± 1	65 ± 2	50 ± 1
9	55 ± 2	44 ± 4	38 ± 1	66 ± 3	69 ± 2
10	40 ± 1	17 ± 4	51 ± 4	65 ± 2	**81 ± 1**
11	42 ± 2	17 ± 4	**70 ± 1**	68 ± 3	56 ± 3
12	38 ± 1	64 ± 3	60 ± 1	63 ± 1	**85 ± 1**
13	44 ± 3	67 ± 4	**82 ± 1**	59 ± 4	49 ± 4
14	62 ± 1	**75 ± 4***	54 ± 3	57 ± 2	50 ± 2
15	64 ± 1	66 ± 3	64 ± 3	66 ± 3	50 ± 4
16	40 ± 1	41 ± 2	68 ± 4	**78 ± 3**	**78 ± 3**
17	28 ± 4	41 ± 2	**71 ± 4**	**80 ± 3**	45 ± 3
18	36 ± 2	23 ± 5	**80 ± 1**	**82 ± 4**	**73 ± 5**
19	46 ± 4	**82 ± 4**	**74 ± 3**	**78 ± 3**	**78 ± 3**
20	56 ± 1	66 ± 3	64 ± 3	**76 ± 3**	**89 ± 1**
21	28 ± 4	35 ± 3	62 ± 1	**78 ± 3**	**85 ± 2**
22	68 ± 1	67 ± 2	34 ± 1	60 ± 1	58 ± 2
23	60 ± 1	50 ± 1	68 ± 4	63 ± 3	**84 ± 1**
24	60 ± 4	44 ± 4	57 ± 1	**86 ± 3**	24 ± 3
25	62 ± 2	15 ± 4	54 ± 3	**89 ± 4**	26 ± 1
26	52 ± 3	44 ± 4	31 ± 1	**76 ± 3**	21 ± 2
27	63 ± 5	16 ± 3	14 ± 3	**83 ± 3**	30 ± 2
28	60 ± 1	50 ± 1	48 ± 4	**80 ± 3**	40 ± 3
T	35 ± 2	**82 ± 5**	30 ± 4	52 ± 2	41 ± 5
V	31 ± 2	35 ± 3	40 ± 4	48 ± 5	**74 ± 1**
C	58 ± 4	**77 ± 5**	68 ± 2	**75 ± 2**	60 ± 4

T: Triadimefon, V: Validamycin, C: Carbendazim, *Bold numbers represent that the efficacies reach grade B.

have good inhibitory effect on *P. piricola*. Compounds with aliphatic substituents (**24-28**) displayed better antifungal activity than compounds with aromatic substituents (**1-23**). **25** ($R = CH_3(CH_2)_2CH_2$) exhibit highest antifungal activity (89 %), much higher than carbendazim (75 %). With increasing or decreasing carbon chains of aliphatic substituents, the antifungal activity decreased gradually. On the other hand, benzene with two positions substituented increased the antifungal activity. **16-21** had much better inhibitory effect than other compounds with aromatic substituents (**1-15, 22** and **23**).

From Table-1, we had also found that compounds with aromatic substituents (**1-23**) had much better inhibitory effects on *R. solani* than compounds with aliphatic substituents (**24-28**). It indicated that the steric effect of benzene ring might be important for the antifungal activity. Among these compounds with aromatic substituents, substituents at position 3 were disfavourable and the substituents were more bulky more disfavourable. **2, 5, 8, 11, 13** and **14** had lower activity (**11 > 2 > 5 > 8 = 14 > 13**). It indicated that steric hindrance at position 3 decreased the activity. In addition, the target compounds bearing electron-withdrawing groups at position 2, 4 of the benzyl displayed higher activity and small size groups would be favourable. For example, **20** ($R = 2,4\text{-}F_2\text{-}C_6H_6\text{-}CH_2$) had the highest activity (89 %), much higher than validamycin

(74 %). It should owe to the two electron-withdrawing groups -F with low steric hindrance at position 2 and 4 of the benzyl. And electron-withdrawing groups at position 4 could increase the activity higher than that at position 2. **6**, **9** and **12** displayed higher activity than **4**, **7** and **10**.

Many compounds displayed good inhibitory effects on *C. beticola* than positive controls and substitutional benzyl increased the activity. For example, the activity of **22** (R = $C_6H_6CH_2$) was much lower than other compounds with substitutional benzyl. The electron-withdrawing groups at position 3 of benzyl increase the activity higher than at position 2 and 4 of benzyl. **13** (R = 3-NO_2-$C_6H_6CH_2$) displayed the highest activity (82 %) possibly related with strong electron-withdrawing -NO_2 at position 3 of benzyl. In addition, **19** (R = 2,6-F_2-$C_6H_6CH_2$) showed strong activity (74 %) and it might be related to -F at position 6 of benzyl.

Most compounds displayed low inhibitory effects on *G. sanbinetti* and *F. oxysporum* but **14** (R = 3-OCH_3-$C_6H_6CH_2$-) and **19** (R = 2,6-F_2-$C_6H_6CH_2$) showed good activity against *G. sanbinetti* (75 and 82 %) comparably with carbendazim and triadimefon, respectively. The substituents of benzyl must play an important role.

On the other hand, it was worthwhile to note that some compounds displayed multiple good antifungal activities. **16**, **20** and **21** showed good antifungal activities against *P. piricola* and *R. solani*. **17** exhibited good antifungal activities against *C. beticola* and *P. piricola*. **18** displayed good antifungal activities against *C. beticola*, *P. piricola* and *R. solani*. **19** even showed good antifungal activities against *G. sanbinetti*, *C. beticola*, *P. piricola* and *R. solani*.

Conclusion

Twenty eight new 1,2,4-triazolo[1,5-α]pyrimidine bearing 1,2,4-triazole heterocycle derivatives were synthesized. Their antifungal activities against *F. oxysporum*, *G. sanbinetti*, *C. beticola*, *P. piricola* and *R. solani* were evaluated. The preliminary results indicate that these novel compounds have good inhibitory effects on *P. piricola* and *R. solani*. Moreover, some compounds displayed multiple good antifungal activities. **19** showed good antifungal activities against *G. sanbinetti*, *C. beticola*, *P. piricola* and *R. solani*. The structure-activity relationships of these compounds were also discussed.

At present we are exploring to introduce 1,3,4-thiadiazole heterocycles into 1,2,4-triazolo[1,5-α]pyrimidine and expect to find novel compounds that have better antifungal activities.

ACKNOWLEDGEMENTS

This work was financed by Key Scientific and Technological Research Project of Educational Commission of Hubei Province of China (No. Z20081701) and Wuhan Municipal Science and Technology Project (No. 200961013418).

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