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Synthesis and biological activities of *N*-(1,2,4-triazole-4-yl)-*N*′-(fluorine-containing-phenyl)carbamimidothioates

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Abstract

A series of *N*-(1,2,4-triazole-4-yl)-*N*′-(fluorine-containing-phenyl)carbamimidothioates **5a–i** were synthesized by reacting 4-amine-1,2,4-trizaole with corresponding aryl isothiocyanates in ethanol at room temperature and, in a subsequent step, with methyl iodide. The antifungal activities of the title compounds against the fungi *Rhizoctonia solan* and *Pyricularia orizae* were screened.

Keywords: Synthesis; Biological activity; *N*-(1,2,4-Triazole-4-yl)-*N*′-(fluorine-containing-phenyl)carbamimidothioates

1. Introduction

Trehalose, which is degraded strictly by trehalase, is regarded as a major resource of blood sugar in insects as well as major storage sugar in fungi. Therefore, trehalase inhibitors may be expected to be potential insecticides or fungicides [1].

Recently, some natural trehalase inhibitors were isolated, such as deoxynojirimycin [2], salbostain [3], validamycins [4], validoxylamines [5], and trehazolin (**1**) [6], with the last one exhibiting the strongest antifungal activities towards the plant pathogenic fungi, *Rhizoctonia solan* and *Pyricularia orizae*.

In the course of screening for novel trehalase inhibitors, we reported recently a new group of compounds (**2**) based on the structural model of trehazolin [7]. These compounds showed positive fungicidal activities toward *P. orizae*. In order to promote the activity and elucidate the structure–activity relationship for such compounds, we designed and synthesized another group of compounds (**5**) by introducing 1,2,4-triazole, an effective unit in fungicides, to the structure (Scheme 1).

2. Results and discussion

The designed compounds were prepared by Scheme 2. The fluorine-containing-phenyl isothiocyanates (**3**) were prepared by treating fluorine-containing-phenyl amines with carbon disulfide, sodium hydroxide and ethyl chloroformate [7].

The fluorine-containing-phenyl isothiocynates (**3**) were treated with 4-amino-1,2,4-triazole in C_2H_5OH at room temperature to give the thioureas (**4**). This is followed by reacting thiourea with methyl iodide in CH_3OH and then ammonia to give the target compound (**5**). Surprisingly, ^{1}H NMR spectra indicated the presence of two isomers **5α** and **5β**.

Compounds **5a–i** were screened for the antifungal activities against the fungi *R. solan* and *P. orizae* at the concentration of 100 and 500 μg/ml by spore germination method [8] (Table 1). The fungicidal data indicated that the toxicity of the compound depends on the number and position of the fluorine atoms on the aryl rings. Those containing three fluorine atoms on the aryl ring had stronger toxicity as a whole. Compounds with *o*-fluorine substituted and without *m*-substituted showed higher activities. The results also indicated that the compounds synthesized in this study were less active to *R. solan* and *P. orizae* than compounds **2** [7]. It means that the introduction of 1,2,4-triazole was unsuccessful.

Due to poor water solubilities of compounds **5a–i**, their inhibitory activities toward trehalase in vitro cannot be determined by the standard method [6].

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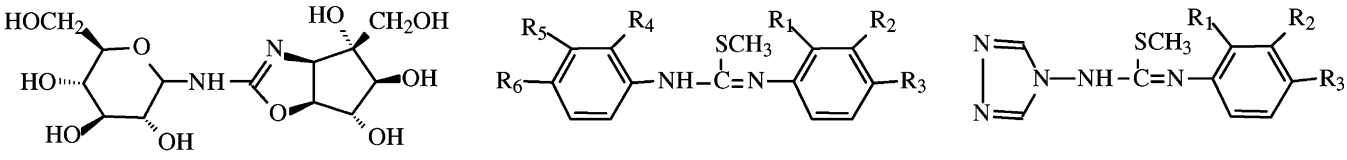


1 **2** **5**

Scheme 1.

3. Experimental

Infrared spectra were taken on a Nicolet FT-IR-20SX spectrometer using KBr disks; mass spectra on a Hitachi M80 instrument; ^{1}H NMR spectra on a Brucker WP100SY (100 MHz) spectrometer with DMSO-6d as solvent and TMS as internal standard. Melting points were measured by a digital melting point apparatus made in Shanghai. Elemental compositions were obtained by using an Italian MOD.1106 analyzer. All reactions were monitored by TLC.

*3.1. Preparation of N-(1,2,4-triazole-4-yl)-N′-(fluorine-containing-phenyl)thioureas (**4**)*

3.1.1. General procedure

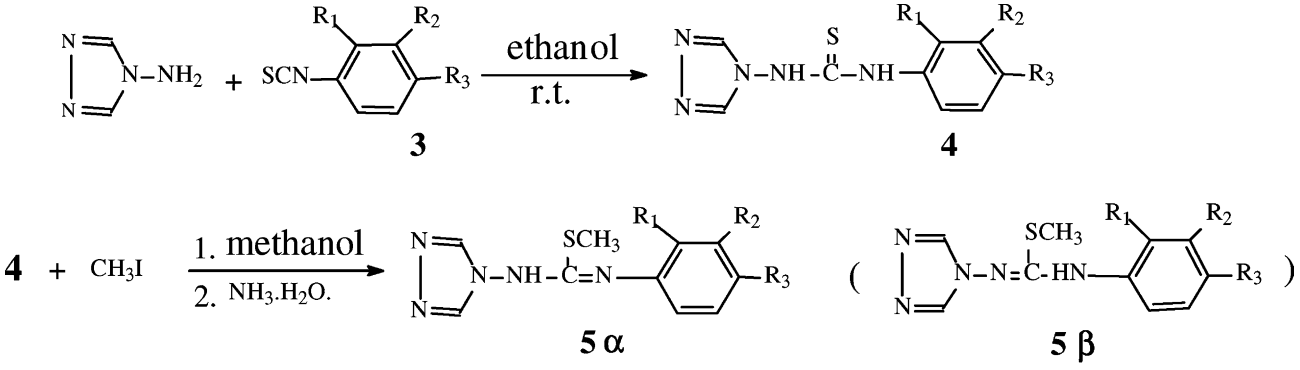
To a solution of 4-amine-1,2,4-trizaole (0.01 mol) in 50 ml of ethanol was added dropwise aryl isothiocyanate (**3**; 0.01 mol) over a period of 10 min. The reaction mixture was stirred for 8 h at room temperature. The solvent was removed under reduced pressure to give crude product, which was recrystallized from ethanol to give a white solid.

*3.2. Preparation of N-(1,2,4-triazole-4-yl)-N′-(fluorine-containing-phenyl)carbamimidothioates (**5**)*

3.2.1. General procedure

To a solution of thiourea (**4**; 0.005 mol) in 50 ml of methanol was added methyl iodide (0.85 g, 0.006 mol). The mixture was heated to reflux for 12 h. The solvent was removed under reduced pressure, and to the residue was added 50 ml of H_2O and 10 ml of concentrated aqueous ammonia. The precipitated product was filtered, washed with H_2O, and recrystallized from C_2H_5OH to give a white solid. The following new compounds were prepared.

Methyl *N*-(1,2,4-triazole-4-yl)-*N′*-(2-fluoro-phenyl)carbamimidothioates (**5a**): yield 65%, mp 218–219 °C. IR (KBr; cm^{-1}): 3120 (NH); 1580 (C=N). ^{1}H NMR (DMSO-6d): 2.48 (s, 1.14H, β-SCH_3), 2.56 (s, 1.86H, α-SCH_3), 7.19–7.58 (m, 4H, α- and β-ArH), 8.42 (s, 1.22H, α-triazole-H), 8.52 (s, 0.78H, β-triazole-H). MS (EI, 70 eV) *m/e* (%): 251 (56) [M], 232 (62) [M–F], 203 (23) [M–CH_3SH], 182 (68) [M–$C_2H_3N_3$], 136 (100) [M–$C_2H_3N_3$–SCH_3], 109 (40) [M–$C_4H_6N_4S$], 83 (50) [M–C_8H_7FNS]. Analysis: Calc.



5a) R_1 = F, R_2 = H, R_3 = H

5b) R_1 = H, R_2 = H, R_3 = F

5c) R_1 = F, R_2 = H, R_3 = F

5d) R_1 = H, R_2 = F, R_3 = F

5e) R1 = F, R2 = F, R3 = F

5f) R_1 =H, R_2 = Cl, R_3 = F

5g) R_1 = H, R_2 = Cl, R_3 = H

5h) R_1 = H, R_2 = H, R_3 = Cl

5i) R_1 = H, R_2 = H, R_3 = H

Scheme 2.

Table 1
Antifungal activity data for compounds **5a–i** and **2a–i**

Compound	*R. solan* (%)		*P. orizae* (%) 100 μg/ml
	500 μg/ml	100 μg/ml	
5a	60.0	8.3	66.7
5b	46.7	0.0	33.3
5c	40.0	0.0	73.3
5d	43.3	0.0	33.3
5e	40.0	0.0	26.7
5f	68.3	58.3	26.7
5g	46.7	0.0	26.7
5h	46.7	0.0	26.7
5i	63.3	9.7	26.7
2a		81.6	100
2b		78.9	100
2c		89.5	100
2d		84.2	100
2e		100	100
2f		77.6	100
2g		92.1	100
2h		38.3	92.3
2i		65.8	100

(**2a**) $R_1 = F$, $R_2 = H$, $R_3 = H$, $R_4 = F$, $R_5 = H$, $R_6 = H$; (**2b**) $R_1 = H$, $R_2 = H$, $R_3 = F$, $R_4 = H$, $R_5 = H$, $R_6 = F$; (**2c**) $R_1 = F$, $R_2 = H$, $R_3 = F$, $R_4 = F$, $R_5 = H$, $R_6 = F$; (**2d**) $R_1 = F$, $R_2 = H$, $R_3 = F$, $R_4 = H$, $R_5 = F$, $R_6 = F$; (**2e**) $R_1 = F$, $R_2 = F$, $R_3 = F$, $R_4 = F$, $R_5 = H$, $R_6 = F$; (**2f**) $R_1 = F$, $R_2 = F$, $R_3 = F$, $R_4 = H$, $R_5 = F$, $R_6 = F$; (**2g**) $R_1 = F$, $R_2 = F$, $R_3 = F$, $R_4 = F$, $R_5 = F$, $R_6 = F$; (**2h**) $R_1 = H$, $R_2 = Cl$, $R_3 = F$, $R_4 = H$, $R_5 = Cl$, $R_6 = F$; (**2i**) $R_1 = F$, $R_2 = H$, $R_3 = Cl$, $R_4 = F$, $R_5 = H$, $R_6 = Cl$.

for $C_{10}H_{10}FN_5S$ (251.2839): C, 47.80; H, 4.01; N, 27.87%. Found: C, 47.81; H, 4.02; N, 27.78%.

Methyl *N*-(1,2,4-triazole-4-yl)-*N*′-(4-fluoro-phenyl)carbamimidothioates (**5b**): yield 68%, mp 228–229 °C. IR (KBr; cm^{-1}): 3120 (NH); 1580 (C=N). ^1H NMR (DMSO-6d): 2.39 (s, 0.79H, β-SCH_3), 2.64 (s, 2.21H, α-SCH_3), 7.16–7.68 (m, 4H, α- and β-ArH), 8.50 (s, 1.46H, α-triazole-H), 8.53 (s, 0.54H, β-triazole-H). MS (EI, 70 eV) *m/e* (%): 251 (84) [M], 203 (30) [M–CH_3SH], 182 (57) [M–$C_2H_3N_3$], 136 (100) [M–$C_2H_3N_3$–SCH_3], 109 (60) [M–$C_4H_6N_4S$], 95 (51) [M–$C_4H_6N_5S$], 83 (55) [M–C_8H_7FNS]. Analysis: Calc. for $C_{10}H_{10}FN_5S$ (251.28): C, 47.80; H, 4.01; N, 27.87%. Found: C, 47.77; H, 4.04; N, 27.83%.

Methyl *N*-(1,2,4-triazole-4-yl)-*N*′-(2,4-difluorophenyl)-carbamimidothioates (**5c**): yield 64%, mp 207–208 °C. IR (KBr; cm^{-1}): 3120 (NH); 1550 (C=N). ^1H NMR (DMSO-6d): 2.40 (s, 1.23H, β-SCH_3), 2.56 (s, 1.77H, α-SCH_3), 7.10–7.60 (m, 3H, α- and β-ArH), 8.41 (s, 1.09H, α-triazole-H), 8.53 (s, 0.91H, β-triazole-H). MS (EI, 70 eV) *m/e* (%): 269 (100) [M], 250 (78) [M–F], 222 (15) [M–CH_3S], 186 (53) [M–$C_2H_4N_4$], 172 (69) [M–$C_2H_4N_4$–CH_3], 154 (96) [M–$C_2H_3N_3$–SCH_3], 127 (55) [M–$C_4H_6N_4S$]. Analysis: Calc. for $C_{10}H_9F_2N_5S$ (269.2714): C, 44.61; H, 3.37; N. 26.01%. Found: C, 44.59; H, 3.36; N, 25.98%.

Methyl *N*-(1,2,4-triazole-4-yl)-*N*′-(3,4-difluoro-phenyl)-carbamimidothioates (**5d**): yield 70%, mp 187–189 °C. IR (KBr; cm^{-1}): 3120 (NH); 1590 (C=N). ^1H NMR (DMSO-6d): 2.43 (s, 0.53H, β-SCH_3), 2.53 (s, 2.47H, α-SCH_3), 7.00–7.84 (m, 3H, α- and β-ArH), 8.54 (s, 2H, α- and β-triazole-H). MS (EI, 70 eV) *m/e* (%): 269 (57) [M], 221 (26) [M–CH_3SH], 200 (55) [M–$C_2H_3N_3$], 186 (41) [M–$C_2H_4N_4$], 172 (47) [M–$C_2H_4N_4$–CH_3], 154 (100) [M–$C_2H_3N_3$–SCH_3], 127 (61) [M–$C_4H_6N_4S$]. Analysis: Calc. for $C_{10}H_9F_2N_5S$ (269.27): C, 44.61; H, 3.37; N, 26.01%. Found: C, 44.60; H, 3.35; N, 26.06%.

Methyl *N*-(1,2,4-triazole-4-yl)-*N*′-(2,3,4-trifluoro-phenyl)carbamimidothioates (**5e**): yield 75%, mp 224–225 °C. IR (KBr; cm^{-1}): 3130 (NH); 1520 (C=N). ^1H NMR (DMSO-6d): 2.43 (s, 0.90H, β-SCH_3), 2.57 (s, 2.10H, α-SCH_3), 7.20–7.39 (m, 2H, α- and β-ArH), 8.43 (s, 1.36H, α-triazole-H), 8.55 (s, 0.64H, β-triazole-H). MS (EI, 70 eV) *m/e* (%): 287 (44) [M], 268 (10) [M–F], 239 (16) [M–CH_3SH], 218 (100) [M–$C_2H_3N_3$], 172 (99) [M–$C_2H_2N_3$–SCH_3], 145 (44) [M–$C_6H_2F_3$]. Analysis: Calc. for $C_{10}H_8F_3N_5S$ (287.26): C, 41.81; H, 2.81; N, 24.38%. Found: C, 41.80; H, 2.79; N, 24.36%.

Methyl *N*-(1,2,4-triazole-4-yl)-*N*′-(3-chloro-4-fluoro-phenyl)carbamimidothioates (**5f**): yield 55%, mp 218–219 °C. IR (KBr; cm^{-1}): 3120 (NH); 1570 (C=N). ^1H NMR (DMSO-6d): 2.43 (s, 0.59H, β-SCH_3), 2.54 (s, 2.41H, α-SCH_3), 7.18–7.99 (m, 3H, α- and β-ArH), 8.55 (s, 2H, α- and β-triazole-H). MS (EI, 70 eV) *m/e* (%): 287 (23) [M + 2], 285 (69) [M], 237 (25) [M–CH_3SH], 202 (54) [M–$C_2H_3N_4$], 170 (100) [M–$C_2H_3N_3$–SCH_3], 143 (52) [M–$C_4H_6N_4S$], 129 (44) [M–$C_4H_6N_5S$]. Analysis: Calc. for $C_{10}H_9ClFN_5S$ (285.73): C, 42.04; H, 3.17; N, 24.51%. Found: C, 42.00; H, 3.15; N, 24.54%.

Methyl *N*-(1,2,4-triazole-4-yl)-*N*′-(3-chloro-phenyl)carbamimidothioates (**5g**): yield 77%, mp 199–201 °C. IR (KBr; cm^{-1}): 3120 (NH); 1570 (C=N). ^1H NMR (DMSO-6d): 2.44 (s, 0.55H, β-SCH_3), 2.54 (s, 2.45H, α-SCH_3), 6.91–7.92 (m, 4H, α- and β-ArH), 8.55 (s, 2H, α- and β-triazole-H). MS (EI, 70 eV) *m/e* (%): 269 (26) [M + 2], 267 (67) [M], 219 (63) [M–CH_3SH], 198 (38) [M–$C_2H_2N_3$], 184 (55) [M–$C_2H_3N_4$], 152 (100) [M–$C_2H_2N_3$–SCH_3], 111 (67) [M–$C_4H_6N_5S$]. Analysis: Calc. for $C_{10}H_{10}ClN_5S$ (267.74): C, 44.86; H, 3.76; N, 26.16%. Found: C, 44.91; H, 3.71; N, 26.12%.

Methyl *N*-(1,2,4-triazole)-*N*′-(4-chloro-phenyl)carbamimidothioates (**5h**): yield 79%, mp 245–246 °C. IR (KBr; cm^{-1}): 3120 (NH); 1570 (C=N). ^1H NMR (DMSO-6d): 2.42 (s, 0.60H, β-SCH_3), 2.51 (s, 2.40H, α-SCH_3), 7.15–7.71 (m, 4H, α- and β-ArH), 8.53 (s, 2H, α- and β-triazole-H). MS (EI, 70 eV) *m/e* (%): 269 (26) [M + 2], 267 (67) [M], 219 (60) [M–CH_3SH], 184 (55) [M–$C_2H_3N_4$], 151 (100) [M–$C_2H_3N_3$–SCH_3], 111 (40) [M–$C_4H_6N_5S$]. Analysis: Calc. for $C_{10}H_{10}ClN_5S$ (267.74): C, 44.86; H, 3.76; N, 26.16%. Found: C, 44.89; H, 3.74; N, 26.15%.

Methyl *N*-(1,2,4-triazole)-*N*′-phenyl-carbamimidothioates (**5i**): yield 83%, mp 204–205 °C. IR (KBr; cm^{-1}): 3120 (NH); 1580 (C=N). ^1H NMR (DMSO-6d): 2.40 (s, 0.77H, β-SCH_3), 2.52 (s, 2.23H, α-SCH_3), 7.11–7.66 (m,

5H, α- and β-ArH), 8.52 (s, 2H, α- and β-triazole-H). MS (EI, 70 eV) *m/e* (%): 233 (76) [M], 185 (30) [M–CH_3SH], 150 (59) [M–$C_2H_3N_3$], 136 (70) [M–$C_2H_2N_4$–CH_3], 118 (98) [M–$C_2H_2N_3$–SCH_3], 77 (100) [M–$C_4H_6N_5S$]. Analysis: Calc. for $C_{10}H_{11}N_5S$ (233.29): C, 51.49; H, 4.75; N, 30.02%. Found: C, 51.47; H, 4.78; N, 30.05%.

4. Note

Spore germination method:

1. Spores were suspended in sterilized water to the concentration of 10^6 spores/ml.
2. The target compounds were added into spore suspension to the concentration of 100 and 500 μg/ml.
3. The mixture of the medicament and the spore suspension was dropped into a sterilize concave slide and then cultured at 25 °C for 24–48 h.
4. The spore germination were then detected by a microscopy to determine the inhibitory rate of target compounds.

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