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Note

Synthesis of 2-(6-fluorobenzothiazole-2′-ylamino)-4-(phenylthioureido)-6-(substituted thioureido)-1,3,5-triazine as antimicrobial agent

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2,4,6-Trichloro-1,3,5-triazine has been reacted selectively with nucleophilic reagents, 6-Fluoro-2-aminobenzothiazole **1**, phenyl thioureas **2** and different substituted thioureas **3** to give 2-(6-fluorobenzothiazole-2′-ylamino)-4-(phenylthioureido)-6-(substituted thioureido)-1,3,5-triazine **4**. These compounds are evaluated for their antimicrobial activity. The structure of all these compounds have been confirmed by IR, ^{1}H NMR, mass spectral data and elemental analysis. Benzothiazoles, *s*-triazenes and thioureas exhibit various biological activities[1].

Keywords: Fluorobenzothiazole, triazine derivatives, antimicrobial activity, antifungal activity, phenyl thioureas, antimicrobial and antifungal activity

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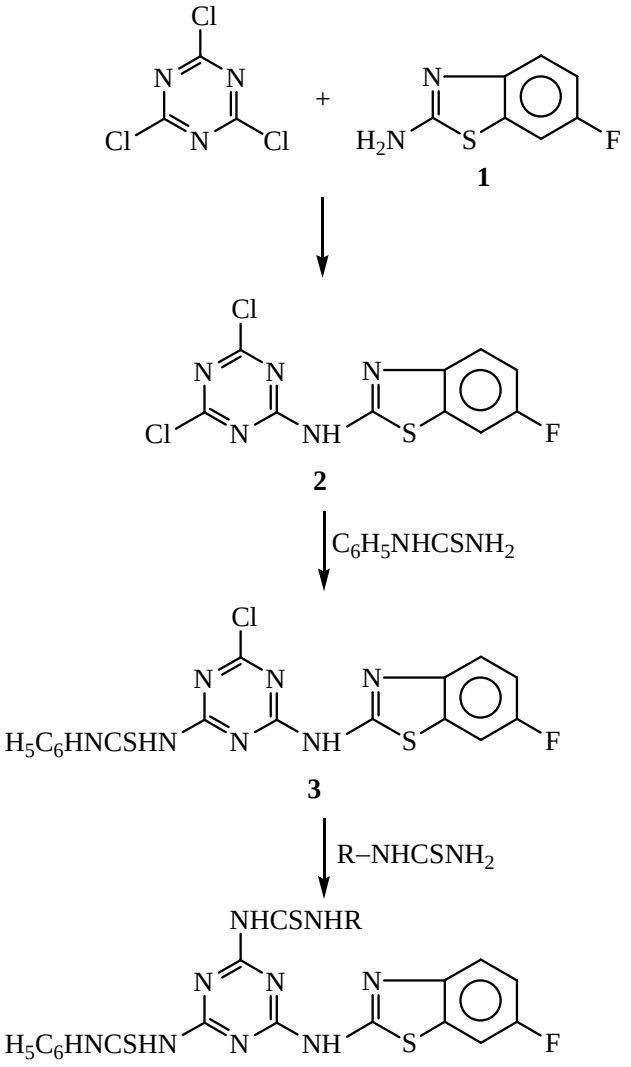
In continuation of our work on triazines[2,3] we have synthesized some new fluorinated derivatives of 2,4,6-trisubstituted 1,3,5-triazine-2-(6-fluorobenzothiazole-2′-ylamino)-4-(phenylthioureido) -6-(substituted thioureido)-1,3,5-triazine **4** (**Scheme I)** with enhanced antimicrobial activity. Three chlorine atoms of 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) have been replaced subsequently by 6-fluoro-2-amino benzothiazole (which in turn is prepared by condensing fluoroaniline with ammonium thiocyanate)[4], phenylthiourea and substituted thioureas in alkaline medium selectively to give the title compound **4**.

2-(6-Fluorobenzothiazole-2′-ylamino)-4,6-dichloro-1,3,5-triazine **2** has been prepared by treating cyanuric chloride in acetone with 6-fluoro-2-amino benzothiazole **1** at 0-5°C and stirring for 3 hr. The second chlorine atom of **2** has been replaced by phenylthiourea at 30-40°C in acetone by constant stirring for 3 hr to give 2-(6-fluorobenzothiazole-2′-ylamino)-4-(phenylthioureido)-6-chloro-1,3,5-triazine **3**. The third chlorine atom of **3** has been replaced by different substituted thioureas at 80-90°C in acetone to give the title compound **4**.

Compound **2** showed the presence of >NH group in both its IR (3150 cm^{-1}) and ^{1}H NMR spectra which showed a broad signal around δ 9.85 (NH). The IR spectrum of 3 showed peak at 1115 cm^{-1} (thioureido CS group) and its ^{1}H NMR spectrum showed a singlet



R = 2-FC_6H_4, 4-FC_6H_4, 2-Cl-5-$CF_3C_6H_3$, 2-ClC_6H_4, 2-$NO_2C_6H_4$, 4-$OCH_3C_6H_4$, 2-$CF_3C_6H_4$, CH_2=CH-CH_2, NH_2, 4-$NO_2C_6H_4$

Scheme I

at δ 4.80 (2H, NHCS NH). Similarly, the absence of a peak at 740 cm^{-1} for chloro group and the presence of a peak at 1110 cm^{-1} for thioureido CS group in compound **4a** revealed that the *p*-fluorophenyl thioureido linkage is present at position 6 of *s*-triazine because out of three chlorine atoms of *s*-triazine ring one is replaced by 2-amino-(6-fluorobenzothiazole) compound **2** and the second one is replaced by phenylthiourea (compound **3**). Thus compound **3** contains only one chlorine atom which should be replaced by *p*-fluorophenylthiourea giving compound **4a**. So it may be inferred that the *p*-fluorophenyl-thiouredo linkage is present at position 6 of *s*-triazine side chain. This was further confirmed by its ^{1}H NMR δ 4.90 (s, 4H, 2×NH CS NH), 10.10 (brs, 1H, NH of benzothiazole) and 6.85-7.45 (m, 12H, Ar-H). Further in the mass spectrum of **4a** molecular ion peak was observed at m/z 565 (M^+).

Fungicidal Activity

Compounds **4a-j** were screened for antifungal activity against *Alternaria alternata, Aspergillus niger* and *Macrophomina* using agar diffusion technique[5]. Culture media were prepared using aseptic[6] and sterilisation[7] techniques. Incubation period is 72 hr at 28°C. All the solutions of test compounds were prepared by dissolving 1 mg of testing sample in 1 mL of acetone. This gives the conc. of sample 1000 μg/mL or 1000 ppm. Different dilutions such as 500 and 100 ppm were prepared from the sample solution. Pure cultures of *Alternaria. alternata, Aspergillus. niger* and *Macrophomina* were raised in conical flask (100 mL) containing potato dextrose agar (PDA) medium. The spores obtained from ten days old cultures were used for testing the efficacy of test compounds against inhibition of spore germination. Sterilized acetone was used as control. Percent spore inhibition is calculated by formula as –

$$\% \text{ Inhibition} = \frac{\text{Number of spores ungerminated}}{\text{Total number of spores}} \times 100$$

Evaluation of the fungicidal activity shows that compounds **4a**, **4b**, **4e** and **4f** showed maximum inhibition which may be attributed to maximum fluoro substitution in compounds **4a**, **4b** and **4e** while in compound **4f** presence of O-CH_3 group along with fluoro group also activates the compound. The fungicidal screening results are recorded in **Table II**.

Experimental Section

Purity of all the compounds was checked on silica gel G plates using iodine vapour as the detecting agent. Melting points were determined in open capillary tubes using Gallen Kamp melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 577 spectrophotometer in KBr pellets. ^{1}H NMR spectra (chemical shifts in δ ppm) were recorded at 89.99 MHz using JEOL (Model FX 90Q) apparatus with TMS as the internal standard. The mass spectra were recorded on Kratos

Table I — Physical and analytical data of the compounds **4a-j**

Compd	R	Mol. Formula	m.p. °C	Yield (%)	Found (Calcd) % C	H	N	S
4a	2-FC_6H_4	$C_{24}H_{17}N_9S_3F_2$	180	66	50.95 (50.97	3.15 3.00	22.34 22.30	17.04 16.99)
4b	4-FC_6H_4	$C_{24}H_{17}N_9S_3F_2$	182	68	50.93 (50.97	3.12 3.00	22.31 22.30	17.02 16.99)
4c	2-ClC_6H_4	$C_{24}H_{17}N_9S_3ClF$	198	70	49.65 (49.61	2.94 2.92	21.74 21.70	16.59 16.55)
4d	2-$NO_2C_6H_4$	$C_{24}H_{17}N_{10}S_3O_2F$	160	65	48.70 (48.64	2.84 2.87	23.68 23.64	16.25 16.55)
4e	2-$CF_3C_6H_4$	$C_{25}H_{15}N_9S_3F_4$	190	67	48.89 (48.93	2.48 2.44	20.59 20.55	15.61 15.66)
4f	4-$OCH_3C_4H_4$	$C_{25}H_{18}N_9OS_3F$	175	69	52.20 (52.17	3.15 3.13	21.95 21.91	16.72 16.69)
4g	2-Cl,4-$CF_3C_6H_3$	$C_{25}H_{15}N_9S_3F_4Cl$	164	63	46.38 (46.33	2.37 2.31	19.51 19.45	14.86 14.82)
4h	CH_2=$CHCH_2$	$C_{21}H_{16}N_9S_3F$	178	60	49.54 (49.50	3.10 3.14	24.81 24.75	18.91 18.86)
4i	NH_2	$C_{18}H_{15}N_{10}S_3F$	140	61	44.48 (44.44	3.12 3.08	28.84 28.80	19.81 19.75)
4j	4-$NO_2C_6H_4$	$C_{24}H_{17}N_{10}S_3O_2F$	170	60	48.68 (48.64	2.84 2.87	23.67 23.64	16.20 16.55)

Table II — Fungicidal screening data of 2-(6-Fluorobenzothiazole-2´ylamino)-4-(phenyl thioureido)-6-(substituted phenyl thioureido) 1,3,5-triazines

Compd	Average % inhibition of spore germination after 72 hr								
	Alternaria alternata Conc. (ppm)			*Aspergillus niger* Conc. (ppm)			*Macrophomina* Conc. (ppm)		
	100	500	1000	100	500	1000	100	500	1000
4a	31	41	68	29	39	67	30	39	70
4b	32	44	66	30	42	66	31	41	68
4c	30	40	56	27	37	55	26	36	54
4d	28	37	55	24	34	50	23	33	49
4e	37	48	70	35	45	69	36	48	71
4f	31	41	62	29	38	60	30	37	64
4g	29	38	59	27	36	58	26	35	60
4h	30	40	60	29	38	51	30	39	59
4i	29	38	56	27	38	52	28	38	56
4j	29	36	56	26	34	53	23	36	50

MS-30 and MS-50 spectrometer operating at an ionization potential of 70 eV.

2-(6-Fluorobenzothiazole-2′-ylamino)-4,6-dichloro-1,3,5-triazine 2. To **2**, 4,6-trichloro-1,3,5-triazine (18.4 g, 0.1 mole) dissolved in acetone (100 mL) cooled at 0°C, 5-fluoro-2-aminobenzothiazole (1.68 g, 0.1 mole) dissolved in acetone (100 mL) was added with stirring at 0-5°C followed by dropwise addition of sodium hydroxide (4.0 g, 0.1 mole) in water (50 mL). Contents were stirred for 3 hr and poured into ice water acidified with dil. HCl, filtered, washed, dried and recrystallised from ethanol, m.p. 240°C, yield (66%); IR (KBr, cm^{-1}): (3150 > NH), ^{1}H NMR ($CDCl_3$): δ 9.85 (s, 1H > NH), 6.5-6.8 (m, 3H, aromatic); MS (m/z): 316 (M^+); (Found: C, 38.25; H, 1.30; N, 22.24; S, 10.21. $C_{10}H_4N_5SFCl_2$ requires C, 38.21; H, 1.27; N, 22.15; S, 10.13%).

2-(6-Fluorobenzothiazole-2′-ylamino)-4-(phenylthioureido)-6-chloro-1,3,5-triazine 3. Compound **2** (31.4 g, 0.1 mole) dissolved in acetone (100 mL) was added to phenylthiourea (1.52 g, 0.1 mole) in acetone (100 mL) slowly with constant stirring followed by the addition of sodium hydroxide (4.0 g, 0.1 mole) in water (50 mL) and stirred for 3 hr at 30-40°C poured in ice water, acidified with dil HCl, filtered washed, dried and recrystallised from ethanol, m.p 210°C, yield 60%. IR (KBr, cm^{-1}): 3130 (>NH), 1115 (thioureido CS), 740 (C-Cl); ^{1}H NMR: δ 4.80 (s, 2H, NHCSNH), 9.75 (s, 1H, >NH), 6.5-7.2 (m, 8H, aromatic); MS (m/z): 399.5 (M^+) (Found: C, 51.10; H, 2.80; N, 24.60; S, 8.95. $C_{17}H_{11}N_7SFCl$ requires C, 51.06; H, 2.75; N, 24.53; S, 8.86%).

2-(6-Fluorobenzothiazole-2′-ylamino)-4-(phenylthioureido)-6-(*p*-fluoro-phenylthioureido)-1,3,5-triazine 4a. To compound **3** (7.43 g, 0.01 mole) in acetone (50 mL) was added 2-fluorophenylthiourea (1.6 g, 0.01 mole) and sodium hydroxide (0.01 mole) in water (10 mL) refluxed at 85-90°C for 2 hr. Then contents were poured into ice water, filtered, dried and recrystallised from ethanol, m.p. 182°C; yield 68 %. IR(KBr, cm^{-1}): 3125 (>NH), 1110 (thioureido CS); ^{1}H NMR: δ 4.90 (s, 4H, 2×NHCSNH), 10.10 (brs, 1H, NH), 6.85-7.45 (m, 12H, Ar-H). MS (m/z): 565 (M^+); (Found: C, 50.93; H, 3.12; N, 22.3, S, 17.02. $C_{24}H_{17}N_9S_3F_2$ requires C, 50.97; H, 3.00; N, 22.30; S, 16.99%).

Compounds **4b-j** were prepared similarly. Their physical and analytical data are recorded in **Table I**.

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