2-Substituted 4H-[1,3]thiazolo[3,2-a][1,3,5]triazine-4-thiones: Synthesis, Crystal Structure, and Antifungal Activity

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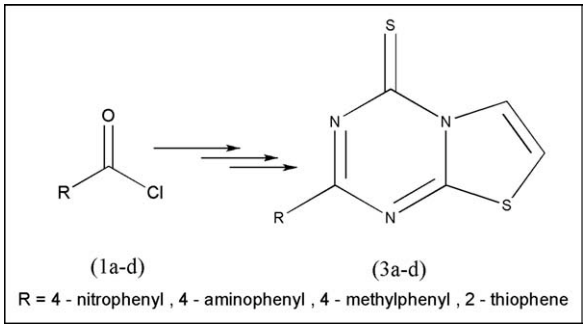

2-(4-Substituted aryl)-4H-[1,3]thiazolo[3,2-a][1,3,5]triazine-4-thiones (**3a-3b**) and 2-(2-thiophene)-4H-[1,3]thiazolo[3,2-a][1,3,5]triazine-4-thione (**3d**) were synthesized by the reaction of arylisothiocyanates/thiophene-2-isothiocyanate with 2-aminothiazole in the presence of tetrabutylammonium bromide as phase transfer catalyst. Compound **3c** was synthesized by the catalytic reduction (10% Pd-C) of **3a**. Compounds **3a–d** were characterized by IR, ^{1}H NMR, ^{13}C NMR, and elemental analysis. All the compounds were tested *in vitro* against *Fusarium solani, A.fumigatus,* and *Aspergillus flavus* using standard drugs. The crystal structure of **3a** was determined from single crystal X-ray diffraction data.

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INTRODUCTION

Heterocyclic compounds containing nitrogen and sulphur possess potential pharmacological activities [1–4]. Recent years have seen a dramatic increase in fungal infections, mostly caused by *Candida albicans*; these infections are often spread through the use of broad-spectrum antibiotics, immunosuppressive agents, anticancer, and anti-AIDS drugs [5]. The main problem in the treatment of fungal infections is the increasing prevalence of drug resistance, especially in patient's chronically subjected to antimycotic therapy such as persons infected with HIV [6]. For these reasons, serious attention has recently been directed toward the discovery and development of new antifungal drugs.

Fused heterocyclic 1,3,5-triazines possess a wide array of biological activities such as herbicidal and fungicidal activity [7], antitumor activity [8], and inhibitory activity against the enzymes phosphodiesterase (PED) [9,10], which is expected to be the target for the treatment of diseases such as asthma, diabetes mellitus, and thrombosis. They are also able to block dihydrofolate reductase, the inhibition of which leads to cell death [11]. The title compounds are examples of such fused heterocyclic 1,3,5-triazines.

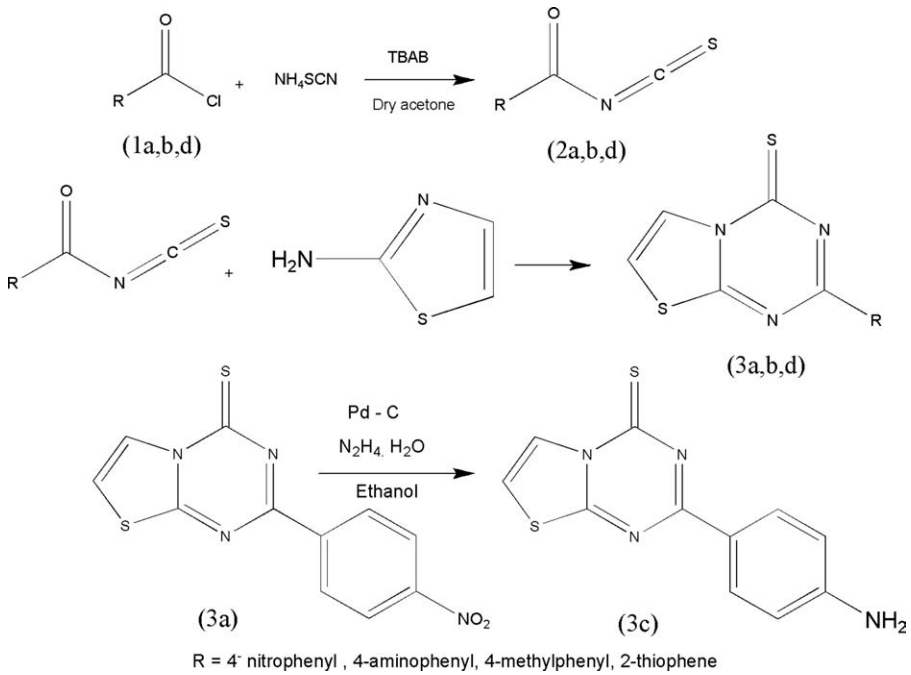
We became interested in the synthesis of fused heterocyclic 1,3,5-triazine compounds containing aryl and thiophene moieties and in the systematic study of their biological activity. All the structures of these novel target compounds were characterized by spectroscopic techniques. We have also confirmed the structure of one representative by X-ray crystallography.

RESULTS AND DISCUSSION

A series of new fused heterocyclic 1,3,5-triazines **3a–d** with thiophene and aroyl substituents (Scheme 1) were prepared by slight modification of published procedures [12,13]. The use of phase transfer catalysts (PTCs) as a method of agitating a heterogeneous reaction system is gaining recognition [14,15]. In search of improved methods to prepare the target fused heterocyclic 1,3,5-triazines by reacting isothiocyanates with nucleophiles, we have found the use of tetrabutylammonium bromide (TBAB) as PTC, which can afford thiophenoyl and aroyl isothiocyanates in good yield, as reported here.

All the structures of newly synthesized compounds were assigned on the basis of their elemental analysis and spectroscopic data, IR, and ^{1}H NMR. All the

Scheme 1. Preparation of compounds (**3a–d**).



compounds were soluble in DMF, DMSO, ethanol, and ethyl acetate.

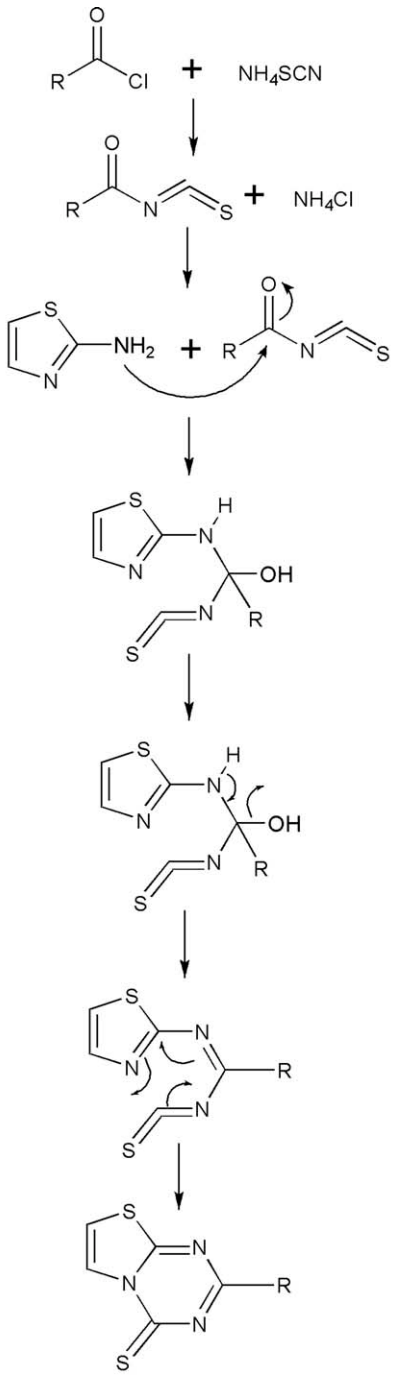
Surprisingly, to the best of our knowledge, 2-(4-substituted phenyl)-4H-[1,3]thiazolo [3,2-a][1,3,5]triazine-4-thiones (**3a–c**) and 2-(2-thiophene)-4H-[1,3]thiazolo [3,2-a][1,3,5]triazine-4-thione (**3d**) have never been described in the literature. The formation of **3a–d** would be explained through the formation of an unstable intermediate form containing thiazole, alcoholic, and isothiocyanate functional groups as shown in Scheme 2. The yield of the target products was very sensitive to the reaction conditions. Two types of products were obtained during the reaction of arylisothiocyanate with 2-aminothiazole. The major product, using a molar ratio of 1:1 between arylisothiocyanate and 2-aminothiazole, was a fused heterocyclic 1,3,5-triazine with about 75% yield and the minor product was a thiourea derivative with about 20% yield. Initially, the experiments were performed three times and these yields remained. However, the yield of fused heterocyclic 1,3,5-triazine products increased to above 90% when the molar concentration of 2-aminothiazole was doubled and refluxed time increased to 4.5 h.

The cyclization of **2a–d** to fused heterocyclic 1,3,5-triazines **3a–d** was monitored by the IR spectra, where the carbonyl chloride peak disappeared on the expense of the appearance of thioamide (C=S) peak in the region of 1445–1440 cm^{-1}. The thioamide group was also confirmed by ^{13}C NMR, with signals at δ 182–179 ppm.

Single crystals of **3a** suitable for X-ray diffraction studies were obtained by evaporation from dichloromethane/ethanol. The molecular structure is shown in Figure 1. Molecular dimensions may be regarded as normal. The bicyclic thiazolotriazine system is planar (mean deviation 0.01 Å) and subtends an interplanar angle of 11.9° to the phenyl ring. The molecular packing (Fig. 2) displays two contacts, H16···O2 2.36 Å and S1···O1 3.01 Å, which combine to form ribbons parallel to the vector [100] and to the plane (012). These ribbons are connected by N1···O2 3.03 Å (not shown).

Primary bioassay screening provides the first indication of bioactivities and helps in the selection of lead compounds for secondary screening for detailed pharmacological evaluation. The synthesized fused heterocyclic 1,3,5-triazines **3a–d** were checked for their antifungal activity against three fungal strains: *Fusarium solani, A.fumigatus,* and *Aspergillus flavus*. The antifungal activity was carried out in DMSO using the agar tube dilution method [16]. Growth in the media was determined by measuring linear growth (mm) and growth inhibition was calculated with reference to the negative control. No significant activity against yeast was detected. All the compounds in the series showed weak antimicrobial activity against *Fusarium solani, A.fumigatus,* and *Aspergillus flavus* with 30–40% inhibition, which shows low activity. However, the compound 2-(2-thiophene)-4H-[1,3]thiazolo [3,2-a][1,3,5]triazine-4-thione (**3d**) showed 40% inhibition.

Scheme 2. Proposed reaction mechanism.



EXPERIMENTAL

Melting points were recorded on Electrothermal IA9000 series digital melting point apparatus. The proton NMR and ^{13}C spectra were recorded in DMSO-d_6 solvent on Bruker 300 MHz spectrophotometer using tetramethylsilane as an internal reference, respectively. The apparent resonance multiplicity is described as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), and m (multiplet). Infrared measurements were recorded in the range 400–4000 cm^{-1} on spectrum 2000 by Perkin Elmer. Elemental analysis was carried out using Perkin Elmer CHNS/O 2400. Obtained results were within 0.4% of the theoretical values. Thin layer chromatography (TLC) analysis were carried out on 5 × 20 cm plate coated with silica gel GF_{254} type 60 (25–250 mesh) using an ethyl acetate-petroleum ether mixture (1:2) as solvent.

2-(4-Nitrophenyl)-4H-[1,3]thiazolo[3,2-a][1,3,5]triazine-4-thione (3a). A solution of 4-nitrobenzoyl chloride (1.85 g, 0.01 mol) in anhydrous acetone (80 mL) and 3% TBAB in acetone was added dropwise to a suspension of ammonium thiocyanate (0.76 g, 0.01 mol) in acetone (50 mL), and the reaction mixture was refluxed for 45 min. After cooling to room temperature, a solution of 2-aminothiazole (1.0 g, 0.01 mol) in acetone (25 mL) was added and the resulting mixture refluxed for 4.5 h. The reaction mixture was poured into five times its volume of cold water, to precipitate the product, which was recrystallized from ethanol: dichloromethane (1:2) as intensely yellow crystals. Yield: 1.50 g (93%), m.p. 196°C; IR (KBr pellet) in cm^{-1}: 1529 (benzene ring), 1512 (NO_2), 1401 (C—N stretching), 1140 (C=S); ^1H NMR (300 MHz, DMSO-d_6) in δ (ppm) and J (Hz): 8.10(2H, d, J = 8.41), 7.34 (2H, d, J = 8.7 Hz); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm): 179.2 (C=S), 165.5 (C), 162.0 (C), 150.4 (C), 145.6 (C), 141.3 (C), 135.1 (C), 128.4 (C) .*Anal*.Calcd. for $C_{11}H_6N_4O_2S_2$ (290.32): C, 45.51; H, 2.08; N, 19.30; S, 22.09. Found: C, 45.50; H, 2.09; N, 19.30; S, 22.07.

2-(4-Methylphenyl)-4H-[1,3]thiazolo[3,2-a][1,3,5]triazine-4-thione (3b). A solution of 4-methylbenzoyl chloride (1.54 g, 0.01 mol) in anhydrous acetone (80 mL) and 3% TBAB in acetone was added dropwise to a suspension of ammonium thiocyanate (0.76 g, 0.01 mol) in acetone (50 mL), and the reaction mixture was refluxed for 45 min. After cooling to room temperature, a solution of 2-aminothiazole (1.0 g, 0.01 mol) in acetone (25 mL) was added and the resulting mixture refluxed for 4.5 h. The reaction mixture was poured into five times its volume of cold water to precipitate the product, which was recrystallized from ethanol as a pale yellow power. Yield: 1.50 g (90%), m.p. 175°C; IR (KBr pellet) in cm^{-1}: 1531 (benzene ring), 1403 (C—N stretching), 1143 (C=S); ^1H NMR (300 MHz, DMSO-d_6) in δ (ppm) and J (Hz): 8.04(2H, d, J = 8.33), 7.24 (2H, d, J = 8.5 Hz), 2.46 (3H,s, —CH_3); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm) : 180.1 (C=S), 165.0 (C), 162.7 (C), 150.4(C), 145.5 (C), 140.7 (C), 135.3 (C), 127.8(C), 25.0 (C). *Anal*.Calcd. for $C_{12}H_9N_3S_2$ (259.35): C, 55.57; H, 3.50; N, 16.20; S, 24.73.Found: C, 55.59; H, 3.52; N, 16.18; S, 24.72.

2-(4-Aminophenyl)-4H-[1,3]thiazolo[3,2-a][1,3,5]triazine-4-thione (3c). Compound **3a** (2.60 g, 0.01 mol), 5 mL hydrazine monohydrate, 70 mL ethanol and 0.03 gm of 10% Pd—C was transferred into 250-mL two-necked round-bottom flask and refluxed for 18 h. The reaction was monitored by TLC. After completion, the reaction mixture was allowed to stand for 1 day and then filtered. The solvent was removed by rotary evaporation. The crude product was recrystallized from ethyl acetate. Yield: 1.50 g (85%), m.p. 165°C; IR (KBr pellet) in cm^{-1}: 1529 (benzene ring), 1405 (C—N stretching), 1140 (C=S); ^1H NMR (300 MHz, DMSO-d_6) in δ (ppm) and J (Hz): 8.10(2H, d, J = 8.41), 7.34 (2H, d, J = 8.7 Hz), 4.01(2H, br s, NH_2); ^{13}C NMR (300 MHz, DMSO-d_6) in

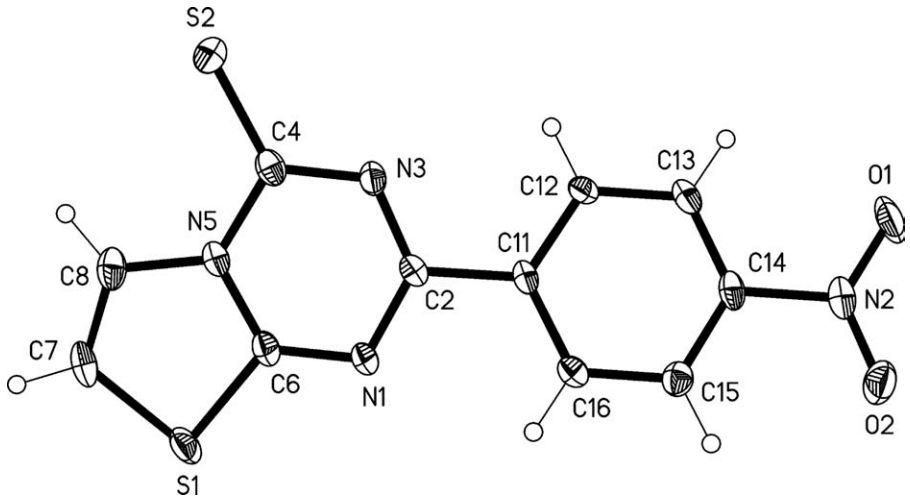


Figure 1. The structure of 2-(4-nitrophenyl)-4H-[1,3]thiazolo[3,2-a][1,3,5]triazine-4-thione (**3a**) with displacement ellipsoids plotted at 50% probability level.

δ (ppm): 180.2 (C=S), 163.3 (C), 162.1 (C), 150.8 (C), 144.6 (C), 140.3 (C), 134.9 (C), 128.7 (C). *Anal*. Calcd. for $C_{11}H_8N_4S_2$ (260.34): C, 50.75; H, 3.10; N, 21.52; S, 24.63. Found: C, 50.78; H, 3.12; N, 21.52; S, 24.64.

2-(2-Thiophene)-4H-[1,3]thiazolo[3,2-a][1,3,5]triazine-4-thione (3d). A solution of thiophene-2-carbonyl chloride (1.46 g, 0.01 mol) in anhydrous acetone (80 mL) and 3% TBAB in acetone was added dropwise to a suspension of ammonium thiocyanate (0.76 g, 0.01 mol) in acetone (50 mL), and the reaction mixture was refluxed for 45 min. After cooling to room temperature, a solution of 2-aminothiazole (1.0 g, 0.01 mol) in acetone (25 mL) was added and the resulting mixture refluxed for 5 h. The reaction mixture was poured into five times its volume of cold water to precipitate the product, which was recrystallized from ethanol as an intense yellow powder. Yield: 1.50 g (93%), m.p. 184°C; IR (KBr pellet) in cm^{-1}: 1402 (C—N stretching), 1144 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6) in δ (ppm) and *J* (Hz): 8.10 (1H, d, $J = 7.2$ Hz, Thiophene CH), 7.91 (1H, dd, $J_1 = 7.5$ Hz, $J_2 = 8.2$ Hz, Thiophene CH), 7.80 (1H, d, $J = 6.7$ Hz, Thiophene CH); ^{13}C NMR (300 MHz, DMSO-d_6) in δ (ppm): 179.2 (C=S), 165.5 (C), 162.0 (C), 150.4 (C) , 145.6 (C), 141.3 (C), 135.1 (C), 128.4 (C) . *Anal*. Calcd. for $C_9H_5N_3S_3$ (251.35): C, 43.01; H, 2.01; N, 16.72; S, 38.27. Found: C, 43.01; H, 2.01; N, 16.72; S, 38.27.

Single crystal X-ray diffraction analysis of 3a. Crystal data: $C_{11}H_6N_4O_2S_2$, orthorhombic, space group *Pbca*, $a = 12.4958(6)$, $b = 8.9836(5)$, $c = 20.5812(12)$ Å, $V = 2310.4(2)$ Å^3, $T = 100$ K, $Z = 8$, F (000) = 1184, $D_x = 1.669$ g cm^{-3}, $\mu = 4.236$ mm^{-1}. Single crystals suitable for X-ray diffraction studies were obtained by evaporation from dichloromethane/ethanol. A yellow plate 0.08 × 0.04 × 0.015 mm^3 was mounted on a glass fiber in inert oil. Measurements were performed at 100 K on an Oxford Diffraction Xcalibur Nova diffractometer with mirror-focused Cu-Kα radiation to $2\theta_{max}$ 152° (99.4% complete to 145°). The data were corrected for absorption using the multiscan method. Of 26,206 intensities, 2367 were independent (R_{int} 0.071). The structure was refined anisotropically using SHELXL-97 [17]. Hydrogen atoms were

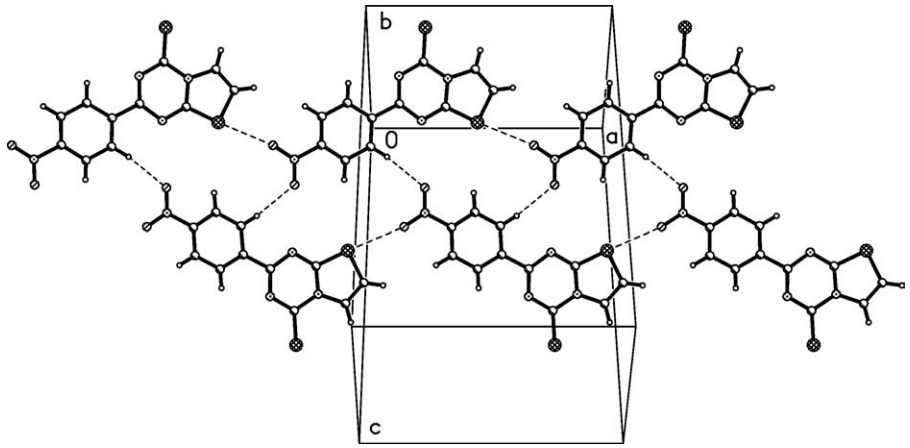


Figure 2. Packing diagram of compound **3a**.

included using a riding model. The final *wR*2 was 0.124, with a conventional *R*1 of 0.046, for 172 parameters; $S = 0.94$; max. $\Delta\rho$ 0.39 e $Å^{-3}$.

CCDC 754654 contains the supplementary crystallographic data for this article. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CBZ IEZ, UK. Facsimile (44) 01223 336 033, E-mail: deposit@ccdc.cam.ac.uk or http//www.ccdc.com.ac.uk/deposit.

ANTIFUNGAL SCREENING

The antifungal activity was carried out in DMSO using the agar tube dilution method. Sabouraud dextrose agar (Merck) was prepared by dissolving 6.5 g/mL in distilled water and the pH was adjusted to 5.6. The contents were dissolved and dispensed in 4-mL aliquots into screw-capped tubes and were autoclaved at 121°C for 21 min. The tubes were allowed to cool to 50°C and nonsolidified SDA was loaded with 66.6 μL of compound by pipette from stock solution, giving a final concentration of 200 μg/mL. The tubes were then allowed to solidify in a leaning position at room temperature. Tubes were prepared in triplicate for each fungus species. The tubes containing solidified media and test compound were inoculated with 4-mm diameter pieces of inocula, taken from a 7-day-old culture of fungus. Other media supplemented with DMSO and nystatin were used as negative and positive control, respectively. The tubes were incubated at 27°C for 7 days. Cultures were examined twice weekly during the incubation. Growth in media was determined by measuring linear growth (mm) and growth inhibition was calculated with reference to the negative control.

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