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RESEARCH ARTICLE

Synthesis, structure, and antifungal evaluation of some novel 1,2,4-triazolylmercaptoacetylthiosemicarbazide and 1,2,4-triazolylmercaptomethyl-1,3,4-thiadiazole analogs

Nalan Terzioğlu Klip[1], Gültaze Çapan[1], Aysel Gürsoy[1], Meltem Uzun[2], and Dilek Satana[2]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul University, Istanbul, Turkey, and* [2]*Department of Microbiology and Clinical Microbiology, Faculty of Medicine, Istanbul University, Istanbul, Turkey*

Abstract

Novel 1-[[4-(4-bromophenyl)-5-(2-furyl)-4*H*-1,2,4-triazole-3-yl]mercaptoacetyl]-4-alkyl/aryl-3-thiosemicarbazides (**5–12**) were synthesized by the reaction of 4-(4-bromophenyl)-5-(2-furyl)-4*H*-1,2,4-triazole-3-ylmercaptoacetylhydrazide (**4**) with substituted isothiocyanates. Cyclodehydration of thiosemicarbazides with concentrated sulfuric acid yielded 2-[4-(4-bromophenyl)-5-(2-furyl)-4*H*-1,2,4-triazole-3-yl]mercaptomethyl-5-alkyl/arylamino-1,3,4-thiadiazoles (**13–17**). The new compounds were evaluated for *in vitro* antifungal activity using the microdilution method. The tested compounds showed varying degrees of activity against *Microsporum gypseum* NCPF-580, *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Candida albicans* ATCC 10231 (MIC 8–4 µg/mL).

Keywords: *Antimicrobial activity; 1,3,4-thiadiazoles; thiosemicarbazides; 1,2,4-triazoles*

Introduction

The increasing clinical importance of drug-resistant bacterial pathogens and opportunistic infections caused by fungi, especially in immunocompromised patients receiving immunosuppressive and cytotoxic therapies, has stimulated the search for new, more effective antibacterial and antifungal agents.

The options for treatment of opportunistic infections are still led by amphotericin B and a few azole (*N*-substituted imidazole and triazole) antifungal agents. Fluconazole, itraconazole, and the newest triazole antifungal agent voriconazole, with a structure related to that of fluconazole and a spectrum of activity comparable to that of itraconazole, are currently being used for the treatment of systemic mycoses (Figure 1)[1]. Due to the adverse effects encountered with currently available drugs, the race to market a new azole continues, and many potent antifungal 1,2,4-triazole compounds have been developed recently[2–4].

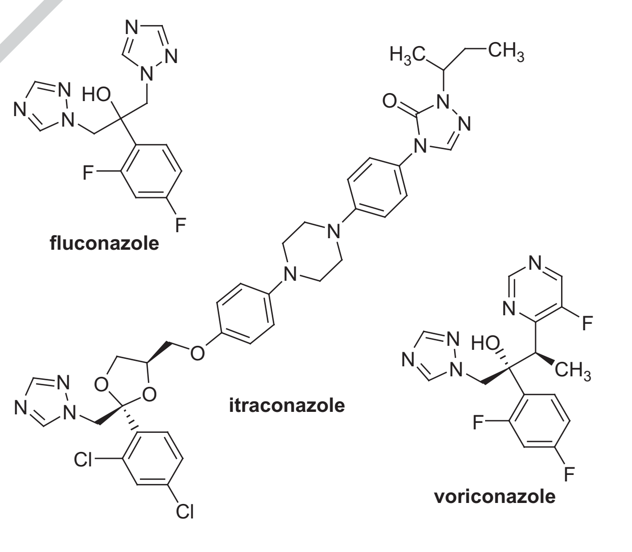

Figure 1. Structures of some antifungal agents.

Address for Correspondence: N. T. Klip, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul University, 34116, Istanbul-Turkey. Tel: +902124400278. Fax: +902124400265. E-mail: nalant@istanbul.edu.tr

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Triazoles cure mycoses by selectively inhibiting fungal cytochrome P-450-mediated lanosterol 14α-demethylase. They prevent demethylation of the natural substrate lanosterol, cause accumulation of 14α-methyl sterols in fungal cells, and decrease ergosterol, which affects membrane structure and functions, resulting in inhibition of the growth of fungi.

Literature surveys reveal that 1,3,4-thiadiazole derivatives as well as 1,2,4-triazoles are associated with antibacterial and antifungal activities[5–9].

In view of the above considerations and in continuation of our previous work on 1,2,4-triazoles and 1,3,4-thiadiazoles[10–15], it was considered worthwhile to design new thiosemicarbazide (**5–12**) and 1,3,4-thiadiazole (**13–17**) derivatives featuring a 1,2,4-triazole nucleus, to investigate their antifungal properties. The present article describes the synthesis and antifungal evaluation of **5–17**.

Materials and methods

2-Furoic acid hydrazide, ethyl bromoacetate, and the isothiocyanates were commercially available. Melting points were determined on a Büchi (Tottoli) apparatus and are uncorrected. Elemental analyses were performed on PerkinElmer 247 and Carlo Erba 1106 elemental analyzers. Ultraviolet (UV; EtOH) and infrared (IR; KBr, cm^{-1}) spectra were run on Shimadzu 2100 S and PerkinElmer 577 (grating) instruments, respectively. ^{1}H-nuclear magnetic resonance (NMR) spectra were recorded on Bruker DPX-400, Bruker AC 200, and Bruker 80 instruments; C_3-H, C_4-H, and C_5-H represent furan protons. Mass spectra (liquid chromatography-mass spectrometry (LC-MS), atmospheric pressure chemical ionization (APCI)) were recorded on a Finnigan LCQ spectrometer in the positive ionization mode.

Compounds **1–4** were prepared as described in previous works[11,16].

*[4-(4-Bromophenyl)-5-(2-furyl)-4H-1,2,4-triazole-3-yl] mercaptoacetic acid hydrazide (**4**)* Yield: 65%, m.p. 171–2°C. IR: 3627, 3567 (N-H); 1671 (C=O). ^{1}H-NMR (400 MHz, $CDCl_3$ + DMSO-d_6): 9.48 (br.s, 1H, NH); 7.66 (d, *J* = 8.6 Hz, 2H, ar); 7.47 (d, *J* = 3.7 Hz, 1H, C_5-H); 7.29 (d, *J* = 8.6 Hz, 2H, ar); 6.37 (dd, *J* = 3.5,1.8 Hz, 1H, C_4-H); 6.23 (d, *J* = 3.5 Hz, 1H, C_3–H); 3.81 (s, 2H, SCH_2); 3.45 (br.s, 1H, NH_2).

General procedure of the synthesis of 1-[[4-(4-bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptoacetyl]-4-alkyl/aryl-3-thiosemicarbazides (5–12)

A solution of **4** (5 mmol) in ethanol (30 mL) and an appropriately substituted isothiocyanate (5 mmol) was heated under reflux for 3–4 h. The prepicitate that formed after cooling was collected by filtration and recrystallized from ethanol (**5–9**, **11**, and **12**) or ethanol/chloroform (3:1) (**10**).

*1-[[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptoacetyl]-4-allyl-3-thiosemicarbazide (**6**)* UV: 275.1 (4.31); 245.8 (4.44). IR: 3330, 3150 (N-H); 1705 (C=O); 1620, 1520, 1485 (C=N, C=C). ^{1}H-NMR (200 MHz, DMSO-d_6): 10.22 (s, 1H, NH); 9.39 (s, 1H, NH); 8.31 (s, 1H, NH); 7.84 (d, *J* = 8.6 Hz, 2H, ar); 7.78 (s, 1H, C_5-H); 7.49 (d, *J* = 8.6 Hz, 2H, ar); 6.55 (dd, *J* = 3.4, 1.7 Hz, 1H, C_4-H); 6.34 (d, *J* = 3.4 Hz, 1H, C_3-H); 5.85 (m, 1H, –CH=CH_2); 5.12 (d, *J* = 17.0 Hz, 1H, =CH_2); 4.62 (dd, *J* = 10.0, 1.4 Hz, 1H,=CH_2); 4.17 (t, 2H, N-CH_2); 3.94 (s, 2H, SCH_2).

*1-[[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptoacetyl]-4-butyl-3-thiosemicarbazide (**7**)* UV: 274.8 (4.29); 244.5 (4.42). IR: 3290, 3140 (N-H); 1705 (C=O); 1540, 1510, 1485 (C=N, C=C). ^{1}H-NMR (200 MHz, DMSO-d_6): 10.12 (s, 1H, NH); 9.21 (s, 1H, NH); 8.06 (t, *J* = 5.4 Hz, 1H, NH); 7.84 (d, *J* = 8.6 Hz, 2H, ar); 7.77 (d, *J* = 1.3 Hz, 1H, C_5-H); 7.49 (d, *J* = 8.6 Hz, 2H, ar); 6.55 (dd, *J* = 3.4, 1.8 Hz, 1H, C_4-H); 6.35 (d, *J* = 4.3 Hz, 1H, C_3-H); 3.89 (s, 2H, SCH_2); 3.51(q, *J* = 6.6 Hz, 2H, N-CH_2); 1.52 (qn, *J* = 7.5 Hz, 2H, CH_2); 1.28 (sx, *J* = 7.3 Hz, 2H, CH_2); 0.87 (t, *J* = 7.2 Hz, 3H, CH_3).

*1-[[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptoacetyl]-4-phenyl-3-thiosemicarbazide (**8**)* UV: 271.8 (4.37); 225.0 (4.41). IR: 3300, 3160 (N-H); 1710 (C=O); 1590, 1540, 1485 (C=N, C=C). ^{1}H-NMR (200 MHz, DMSO-d_6): 10.31 (s, 1H, NH); 9.66 (s, 2H, NH); 7.83 (d, *J* = 8.6 Hz, 2H, ar); 7.77 (d, *J* = 1.3 Hz, 1H, C_5-H); 7.53 (t, *J* = 8.3 Hz, 4H, ar); 7.34 (t, *J* = 7.9 Hz, 2H, ar); 7.16 (t, *J* = 7.3 Hz, 1H, ar); 6.55 (dd, *J* = 3.4, 1.8 Hz, 1H, C_4-H); 6.35 (d, *J* = 3.4 Hz, 1H, C_3-H); 3.97 (s, 2H, SCH_2).

*1-[[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptoacetyl]-4-(4-bromophenyl)-3-thiosemicarbazide (**9**)* UV: 273.5 (4.44); 226.0 (4.48). IR: 3360, 3200 (N-H); 1705, 1695 (C=O); 1585, 1540, 1480 (C=N, C=C). ^{1}H-NMR (200 MHz, DMSO-d_6): 10.41 (s, 1H, NH); 9.89 (s, 1H, NH); 9.79 (s, 1H, NH); 7.85 (d, *J* = 8.6 Hz, 2H, ar); 7.80 (d, *J* = 1.5 Hz, 1H, C_5-H); 7.56–7.50 (m, 6H, ar); 6.56 (dd, *J* = 3.4, 1.8 Hz, 1H, C_4-H); 6.32 (d, *J* = 3.4 Hz, 1H, C_3-H); 3.95 (s, 2H, SCH_2).

*1-[[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptoacetyl]-4-(4-chlorophenyl)-3-thiosemicarbazide (**10**)* UV: 274.0 (4.45); 228.0 (4.52). IR: 3330, 3150 (N-H); 1705 (C=O); 1620, 1520, 1485 (C=N, C=C). ^{1}H-NMR (200 MHz, DMSO-d_6): 10.40 (s, 1H, NH); 9.89 (s, 1H, NH); 9.81 (s, 1H, NH); 7.85 (d, *J* = 8.5 Hz, 2H, ar); 7.80 (d, *J* = 1.6 Hz, 1H, C_5-H); 7.41–7.54 (m, 4H, ar); 7.39 (d, *J* = 8.6 Hz, 2H, ar); 6.55 (dd, *J* = 3.5, 1.8 Hz, 1H, C_4-H); 6.31 (d, *J* = 3.5 Hz, 1H, C_3-H); 3.94 (s, 2H, SCH_2).

*1-[[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptoacetyl]-4-(4-fluorophenyl)-3-thiosemicarbazide (**11**)* UV: 268.6 (4.48); 226.8 (4.56). IR: 3340, 3200 (N-H); 1710, 1685 (C=O); 1605, 1520, 1500, 1480 (C=N, C=C). ^{1}H-NMR (200 MHz, DMSO-d_6): 10.32 (s, 1H, NH); 9.72 (s, 1H, NH); 9.69 (s, 1H, NH); 7.84 (d, *J* = 8.6 Hz, 2H, ar); 7.77 (s, 1H, C_5-H); 7.55–7.49 (m, 4H, ar); 7.17 (t, *J* = 5.8 Hz, 2H, ar); 6.55 (dd, *J* = 3.5, 1.8 Hz, 1H, C_4-H); 6.34 (d, *J* = 3.5 Hz, 1H, C_3-H); 3.96 (s, 2H, SCH_2).

*1-[[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptoacetyl]-4-(4-nitrophenyl)-3-thiosemicarbazide (**12**)* UV: 384.0 (4.01); 278.0 (4.42); 228.0 (4.48). IR: 3310, 3250 (N-H); 1717 (C=O); 1616, 1564, 1512, 1492 (C=N, C=C). ^{1}H-NMR (200 MHz, DMSO-d_6): 10.47 (s, 1H, NH); 10.16 (s, 1H, NH); 10.07 (s, 1H, NH); 8.21 (d, *J* = 9.1 Hz,

2H, ar); 7.98 (d, J = 8.8 Hz, 2H, ar); 7.84 (d, J = 8.5 Hz, 2H, ar);7.78 (s, 1H, C_5-H); 7.52 (d, J = 8.8 Hz, 2H, ar); 6.55 (dd, J = 3.3, 1.7 Hz, 1H, C_4-H); 6.34 (d, J = 3.4 Hz, 1H, C_3-H); 3.97 (s, 2H, SCH_2).

General procedure of the synthesis of 2-[4-(4-bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptomethyl-5-alkyl/aryl-amino-1,3,4-thiadiazoles (13–17)

An appropriate thiosemicarbazide (**5–12**) (2 mmol) was added portionwise to H_2SO_4 (96%) (5.3 mL) cooled in an ice bath with constant stirring. After dissolution, the reaction mixture was further agitated for 30 min at room temperature, poured over crushed ice, and neutralized by the addition of saturated Na_2CO_3 solution. The prepicitate thus obtained was collected by filtration, washed with water, and recrystallized from ethanol (**13**, **14**, **16**, and **17**) or ethanol/chloroform (3:1) (**15**).

*2-[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptomethyl-5-ethyl-amino-1,3,4-thiadiazole (**13**)* UV: 278.2 (4.37); 224.4 (4.26). IR: 3280 (N-H); 1615, 1580, 1510, 1480 (C=N, C=C). ^{1}H-NMR (200 MHz, $CDCl_3$): 7.64 (d, J = 8.7 Hz, 2H, ar); 7.38 (d, J = 1.2 Hz, 1H, C_5-H); 7.15 (d, J = 8.7 Hz, 2H, ar); 6.43 (d, J = 3.5 Hz, 1H, C_4-H); 6.38 (dd, J = 3.6, 1.9 Hz, 1H, C_3-H); 5.70 (s, 1H, NH);4.65 (s, 2H, SCH_2); 3.33 (q, J = 6.8 Hz, 2H, CH_2); 1.24 (t, J = 6.8 Hz, 3H, CH_3). MS-APCI (*m/z*, %): 463 (MH^+, 49), 465 (MH + 2, 100).

*2-[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptomethyl-5-butyl-amino-1,3,4-thiadiazole (**14**)* UV: 279.0 (4.46); 225.0 (4.33). IR: 3210 (N-H); 1610, 1580, 1515, 1480 (C=N, C=C). ^{1}H-NMR (200 MHz, $CDCl_3$): 7.65 (d, J = 8.5 Hz, 2H, ar); 7.39 (s, 1H, C_5-H); 7.15 (d, J = 8.6 Hz, 2H, ar); 6.43 (d, J = 3.5 Hz, 1H, C_4-H); 6.38 (d, J = 3.6 Hz, 1H, C_3-H); 5.43 (s, 1H, NH); 4.66 (s, 2H, SCH_2); 3.28 (t, J = 6.9 Hz, 2H, CH_2); 1.62 (qn, J = 6.9 Hz, 2H, CH_2); 1.38 (sx, J = 6.9 Hz, 2H, CH_2); 0.92 (t, J = 6.9 Hz, 3H, CH_3).

*2-[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptomethyl-5-(4-chlorophenyl)-amino-1,3,4-thiadiazole (**15**)* UV: 287.2 (4.08). IR: 3285 (N-H); 1600, 1545, 1490 (C=N, C=C). ^{1}H-NMR (200 MHz, DMSO-d_6): 10.37 (s, 1H, NH); 7.78 (d, J = 8.0 Hz, 3H, C_5-H, ar); 7.62 (d, J = 8.0 Hz, 2H, ar); 7.40 (t, J = 8.0 Hz, 4H, ar); 6.55 (dd, J = 3.4, 1.7 Hz, 1H, C_4-H); 6.37 (d, J = 3.5 Hz, 1H, C_3-H); 4.65 (s, 2H, SCH_2).

*2-[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl]mercaptomethyl-5-(4-fluorophenyl)-amino-1,3,4-thiadiazole (**16**)* UV: 283.7 (4.39). IR: 3280 (N-H); 1620, 1550, 1510, 1490 (C=N, C=C). ^{1}H-NMR (80 MHz, DMSO-d_6): 7.84–7.05 (m, 9H, C_5-H, ar); 6.55 (dd, J = 3.5, 1.8 Hz, 1H, C_4-H); 6.34 (d, J = 2.8 Hz, 1H, C_3-H); 4.64 (s, 2H, SCH_2).

*2-[4-(4-Bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptomethyl-5-(4-nitrophenyl)-amino-1,3,4-thiadiazole (**17**)* UV: 355.0 (4.32); 278.0 (4.23); 228.0 (4.31). IR: 3261 (N-H); 1615, 1568, 1508, 1487 (C=N, C=C). ^{1}H-NMR (200 MHz, DMSO-d_6): 10.99 (s, 1H, NH); 8.23 (d, J = 9.2 Hz, 2H, ar); 7.80–7.76 (m, 5H, C_5-H, ar); 7.42 (d, J = 8.4 Hz, 2H, ar); 6.54 (dd, J = 3.4, 1.8 Hz, 1H, C_4-H); 6.35 (d, J = 3.4 Hz, 1H, C_3-H); 4.69 (s, 2H, SCH_2). MS-APCI (*m/z*, %): 556 (MH^+, 91), 558 (MH + 2, 100).

Antifungal activity

The microdilution method was used according to a standard protocol of the National Committee for Clinical Laboratory Standards (NCCLS)[17,18]. Five strains were tested from each of the following species: *Microsporum gypseum* NCPF-580, *Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, and *Candida albicans* ATCC 10231.

The medium used was RPMI 1640 broth with L-glutamine and 0.165 M MOPS buffer (3-(*N*-morpholino)propanesulfonic acid; 34.54 g/L) and without sodium bicarbonate. The medium was adjusted to pH 7.0 at 25°C. Sterility control of each bottle was performed before it was used.

Itraconazole (ITC) was provided by the manufacturer as a standard powder. All compounds were dissolved in 100% dimethyl sulfoxide (DMSO) according to NCCLS methods[17,18]. The final compound concentrations were 32–0.01 μg/mL.

Preparation of inoculum suspensions was based mainly on NCCLS guidelines[19] and was described previously[20]. The isolates were subcultured onto potato dextrose agar (PDA) plates at 28°C, during 7–14 days. The fungal colonies were covered with 1 mL of sterile 0.85% saline, and suspensions were made by gently probing the surface with the tip of a Pasteur pipette. The resulting mixture of conidial and hyphal fragments was withdrawn and transferred to a sterile tube. Heavy particles were allowed to settle for 15–20 min at room temperature; the upper suspension was mixed with a vortex for 15 s. The turbidity of supernatants was measured spectrophotometrically at a wavelength of 530 nm, and transmission was adjusted to 65–75%. These stock suspensions were diluted 1:50 in RPMI medium to obtain the final inoculum sizes ranging from 0.4×10^4 to 5×10^4 CFU/mL.

Preparation of inoculum suspensions of *C. albicans* was based mainly on NCCLS guidelines and was described previously[19]. *C. albicans* colonies grown on Sabouraud dextrose agar for 24 h at 35°C were suspended in 5 mL of sterile 0.85% saline. The turbidity of the mixed suspension was measured at 530 nm to obtain 75–77% transmission and adjusted to 1×10^6–5×10^6 CFU/mL by a spectrophotometric method. These stock suspensions were diluted 1:50 in RPMI medium, and further diluted 1:20 with medium to obtain the two-fold test inoculum (1×10^3–5×10^3 CFU/mL). The (two-fold) inoculum was diluted 1:1 when wells were inoculated, and the desired final inoculum size was achieved (0.5×10^3–2.5×10^3 CFU/mL).

Microdilution plates (96 U-shaped) were prepared and frozen at –70°C until needed. Rows from 2 to 12 contained the series of drug dilutions in 100 μL volumes and the first row contained 100 μL of drug-free medium, which served as the growth control. Each well was inoculated on the day of the test with 100 μl of the corresponding inoculum. Microplates of dermatophytes were incubated at 28°C for

7 days and microplates of *C. albicans* were incubated at 35°C, 24 and 48 h. The microplates were read visually with the aid of an inverted reading mirror after 7 days for dermatophytes and after 24 and 48 h for *C. albicans*. For all drugs, except itraconazole, the minimum inhibitory concentration (MIC) was the lowest concentration showing 100% growth inhibition, and the MIC value of itraconazole was determined according to the NCCLS M27-A2 as 80% inhibition.

Results and discussion

The synthetic approach utilized for the required compounds **1–17** is outlined in Scheme 1. The preparation of the key intermediate [4-(4-bromophenyl)-5-(2-furyl)-4*H*-1,2,4-triazole-3-yl]mercaptoacetic acid hydrazide (**4**) was achieved in four steps starting from furoic acid hydrazide, employing a previously described methodology[11,16]. Reaction of **4** with appropriately substituted isothiocyanates afforded corresponding 1-[[4-(4-bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl] mercaptoacetyl]-4-alkyl/aryl-3-thiosemicarbazides (**5–12**), which in turn were treated with concentrated H_2SO_4 at 0–4°C to yield 2-[4-(4-bromophenyl)-5-(2-furyl)-1,2,4-triazole-3-yl]mercaptomethyl-5-alkyl/arylamino-1,3,4-thiadiazoles (**13–17**) (Scheme 1). The structures of **5–17** were established by elemental analysis (Table 1) and spectral data (UV, IR, ^{1}H-NMR, LC-MS).

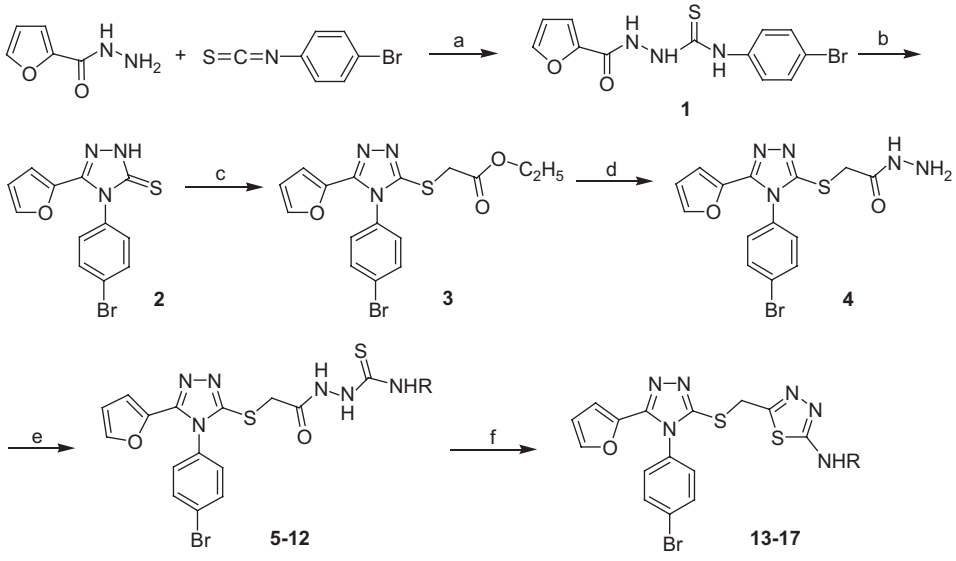
The IR spectra of the thiosemicarbazide derivatives **5–12** showed two N-H bands in the 3360–3290 and 3250–3140 cm^{-1} regions. The C=O stretchings were observed at about 1717–1685 cm^{-1}. Supportive data were obtained from the ^{1}H-NMR spectra of **5–12** as they showed N_1-H, N_2-H, and N_4-H resonances in the δ 10.41–8.06 ppm region[21]. Singlets observed at about δ 3.97–3.87 ppm were attributed to the SCH_2 protons[12].

Cyclodehydration of 1-acyl or 1-aroyl-3-thiosemicarbazides in the presence of a strong base or a strong mineral acid leads to 2,4-dihydro-3*H*-1,3,4-triazole-3-thiones or 5-amino-1,3,4-thiadiazoles. An explanation for the different modes of cyclization is provided by consideration of the relative nucleophilicities of the terminal thioamide function. In a base the sulfur function is ionized, which increases the nucleophilicity of the 4-amino group leading to 1,2,4-triazole formation, whereas in a strong acid the 4-amino group is protonated and cannot participate in the condensation and this leads to 1,3,4-thiadiazole formation[22].

The cyclization reaction proceeds via enol and enethiol intermediates formed by participation of the N_1-H, N_2-H, C=O, and C=S functions of the thiosemicarbazides. Thus, the absence of C=O stretchings in the IR spectra, observation of a single NH resonance (δ 10.99–5.43 ppm)[11] assigned to the proton of the 5-alkylamino group, and downfield shift of the SCH_2 resonances (δ 4.69–4.64 ppm) when compared to those of the thiosemicarbazides (δ 3.97–3.89 ppm) **5–12** in the ^{1}H-NMR spectra of **13–17** provided confirmatory evidence of the formation of expected 1,3,4-thiadiazoles. Further spectral details supporting the structure of the cyclic products are presented in "Materials and methods."

Abundant $(MH)^+$ and $(MH + 2)^+$ ions observed in the APCI (+) mass spectra of **13** and **17** (**13**: **(**$MH)^+$ *m/z* 463, $(MH + 2)^+$ *m/z* 465; **17**: **(**$MH)^+$ *m/z* 556, $(MH + 2)^+$ *m/z* 558) confirmed their molecular weights.

In line with literature findings, selected compounds (**4**, **6**, **7**, **9**, **11–17**) were tested for antifungal activity against *Microsporum gypseum* NCPF-580, *Microsporum canis*, *Tricophyton mentagrophytes*, *Tricophyton rubrum*, and *Candida albicans* ATCC 10231 by microdilution assay using itraconazole as the standard. As can be seen in Table 2, the



Scheme 1. Reagents and conditions: (a) EtOH, reflux, 2 h; (b) 1M NaOH, reflux, 3 h, 1 M HCl; (c) $BrCH_2COOEt$, KOH, EtOH, reflux, 2h; (d) $H_2NNH_2.H_2O$, EtOH, reflux, 3 h; (e) RNCS, EtOH, reflux, 3–4 h; (f) (i) conc. H_2SO_4, 0–4°C, (ii) Na_2CO_3.

Table 1. Physicochemical data of compounds **5–17**.

Compound	R	Formula (MW)	M.p. (°C)	Yield (%)	Analysis (calc./found)		
					C	H	N
5	C_2H_5	$C_{17}H_{17}BrN_6O_2S_2$ (481.38)	212	67	42.41 41.82	3.56 3.43	17.46 17.60
6	C_3H_5	$C_{18}H_{17}BrN_6O_2S_2$ (493.39)	207–209	40	43.81 44.26	3.47 3.70	17.03 16.65
7	C_4H_9	$C_{19}H_{21}BrN_6O_2S_2$ (509.43)	190	71	44.79 45.41	4.15 4.23	16.49 16.68
8	C_6H_5	$C_{21}H_{17}BrN_6O_2S_2$ (529.42)	194–196	84	47.63 47.51	3.23 3.22	15.87 15.71
9	4-BrC_6H_4	$C_{21}H_{16}Br_2N_6O_2S_2.1½H_2O$ (626.34)	195	88	39.70 39.50	3.01 2.70	13.21 13.10
10	4-ClC_6H_4	$C_{21}H_{16}BrClN_6O_2S_2$ (563.86)	183–185	76	44.72 45.59	2.86 2.96	14.90 14.71
11	4-FC_6H_4	$C_{21}H_{16}BrFN_6O_2S_2$ (547.41)	190	71	46.07 45.43	2.94 2.80	15.35 14.66
12	4-$NO_2C_6H_4$	$C_{21}H_{16}BrN_7O_4S_2$ (574.43)	200–202 (dec)	89	43.91 43.43	2.81 2.71	17.07 16.32
13	C_2H_5	$C_{17}H_{15}BrN_6OS_2$ (463.36)	158–159	81	44.06 44.18	3.26 3.29	18.13 18.25
14	C_4H_9	$C_{19}H_{19}BrN_6OS_2$ (491.41)	169–170	87	46.43 46.75	3.89 3.94	17.10 17.21
15	4-ClC_6H_4	$C_{21}H_{14}BrClN_6OS_2$ (545.84)	251–253	86	46.20 47.11	2.58 2.76	15.39 16.16
16	4-FC_6H_4	$C_{21}H_{14}BrFN_6OS_2$ (529.39)	185	80	47.64 47.33	2.66 2.82	15.87 15.38
17	4-$NO_2C_6H_4$	$C_{21}H_{14}BrN_7O_3S_2.2H_2O$ (592.44)	236 (dec)	92	42.57 42.30	3.06 2.30	16.55 16.12

Table 2. Antifungal activity of compounds **4**, **6**, **7**, **9**, **11–17** (MIC: μg/mL)[a].

Compound	R	Microorganism[b]				
		A	B	C	D	E
4	—	8	8	4	8	8
6	C_3H_5	8	8	8	8	8
7	C_4H_9	8	8	4	8	8
9	C_6H_4Br (p)	8	8	4	4	8
11	C_6H_4F (p)	8	8	4	8	8
12	$C_6H_4NO_2$ (p)	8	8	4	8	4
13	C_2H_5	8	8	4	8	8
14	C_4H_9	8	8	4	8	8
15	C_6H_4Cl (p)	8	8	8	8	>32
16	C_6H_4F (p)	8	8	4	8	8
17	$C_6H_4NO_2$ (p)	8	8	4	8	>8
Itraconazole	—	0.03	0.03	0.015<	0.015<	0.015<

[a]MIC value was determined by methods of NCCLS. Concentration range was between 32 and 0.01 μg/mL.
[b]Tested microorganisms: A, *Microsporum gypseum* NCPF-580; B, *Microsporum canis*; C, *Trichophyton mentagrophytes*; D, *Trichophyton rubrum*; E, *Candida albicans* ATCC 10231.

tested compounds inhibited the growth of fungal strains responsible for mycoses at varying concentrations (MIC 8–4 μg/mL). Both aliphatic and aromatic substituents were tolerated, and potency was not altered except in the case of compounds **15** and **17**. The most susceptible microorganism was *T. mentagrophytes*, and two thiosemicarbazide derivatives **9** and **12** demonstrated a wider spectrum of activity with lower MIC values (Table 2). Although no apparent structure–activity relationship can be derived from the above considerations, it may be speculated that the compounds have some potential which deserves further attention for the development of new antifungal candidates.

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