

Synthesis and antimicrobial activity of new 1,2,4-triazole and 1,3,4-thiadiazole derivatives

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(Received 5 January 2007; accepted 28 February 2007)

Abstract

In this study, new 3-[[1(2H)-phthalazinone-2-yl(methyl/ethyl)-4-aryl-1,2,4-triazole-5-thione and 2-[[1(2H)-phthalazinone-2-yl]methyl/ethyl]-5-arylamino-1,3,4-thiadiazole derivatives were synthesized. Antimicrobial properties of the title compounds were investigated against two Gram (+) bacteria (*S. aureus*, *B. subtilis*), two Gram (–) bacteria (*P. aeruginosa*, *E. coli*) and two yeast-like fungi (*C. albicans* and *C. parapsilosis*) using the broth microdilution method. Generally the compounds were found to be active against *B. subtilis* and the fungi. Derivatives carrying a 1,3,4-thiadiazole ring generally showed higher antimicrobial activity against *B. subtilis* and the fungi when compared to other synthesized compounds.

Keywords: Phthalazinone, 1,2,4-triazole, 1,3,4-thiadiazole, antimicrobial activity

Introduction

Since resistance of pathogenic bacteria towards available antibiotics is rapidly becoming a major world-wide problem, the design of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today. In addition, it is known that antifungal drugs do not have selective activity because of the biochemical similarity between human cell and fungi forms. Therefore, there are many studies focused on antibacterial and antifungal compounds [1–3].

Some compounds bearing a 1,2,4-triazole and 1,3,4-thiadiazole structure have been reported to have antimicrobial activity [4–8]. In addition, many compounds carrying a phthalazinone ring have been reported to have diverse biological activities such as antimicrobial [9,10], antihypertensive [11], analgesic and anti-inflammatory activities [12].

In the design of new compounds, development of hybrid molecules through the combination of different pharmacophores in one structure may lead

to compounds with increased antimicrobial activity. Therefore, these observations prompted us to synthesize new 1,2,4-triazole and 1,3,4-thiadiazole derivatives which were attached to position-2 of the phthalazinone ring through a methylene or ethylene bridge. Then, the synthesized compounds were tested against two Gram (+) bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two Gram (–) bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) and two yeast-like fungi *Candida albicans* and *Candida parapsilosis* using the broth microdilution method.

Experimental

Chemistry

All the chemicals used for the synthesis of the compounds were purchased from either Aldrich Chemicals or Merck AG. Melting points were determined with an Electrothermal-9300 digital melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 1330 IR

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spectrophotometer and $^1\text{H-NMR}$ spectra on a Mercury 400 FT NMR spectrometer using tetramethylsilane as the internal standard and DMSO-d_6 as solvent. All chemical shifts were recorded as δ (ppm). Elemental analyses were performed with CHNS-932 (Leco) at Ankara University, Faculty of Pharmacy.

General procedure for [1(2H)-phthalazinone-2-yl]acetyl/propanoylthiosemicarbazides (6a-l). To a solution of the [1(2H)-phthalazinone-2-yl]acetyl-hydrazine (0.0025 mol) in 50 mL methanol or the 3-[1(2H)-phthalazinone-2-yl]propanoylhydrazine (0.0025 mol) in 50 mL ethanol were added the appropriate isothiocyanate derivatives (0.0026 mol) and then each reaction mixture was refluxed until the starting material had been consumed. At the end of this period the precipitating product was filtered and crystallized from an appropriate solvent.

6a. IR (KBr) ν_{max} (cm^{-1}) 1675, 1647; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 10.41 (1H, s, CONH), 9.78 (1H, s, NHNHCSNH), 9.51 (1H, s, NHNHCSNH), 8.47 (1H, s, H^4), 8.22 (1H, d, H^8), 7.97-7.88 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.50 (2H, d, phenyl- $\text{H}^{2,6}$), 7.34 (2H, t, phenyl- $\text{H}^{3,5}$), 7.16 (1H, t, phenyl- H^4), 4.90 (2H, s, CH_2).

6b. IR (KBr) ν_{max} (cm^{-1}) 1679, 1656; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 10.23 (1H, s, CONH), 9.52 (1H, s, NHNHCSNH), 8.45 (1H, s, NHNHCSNH), 8.42 (1H, s, H^4), 8.15 (1H, d, H^8), 7.95-7.84 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.27-7.26 (5H, m, phenyl-H), 4.85 (2H, s, CH_2), 4.74 (2H, d, $\text{CH}_2\text{C}_6\text{H}_5$).

6c. IR (KBr) ν_{max} (cm^{-1}) 1685, 1650; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 10.22 (1H, s, CONH), 9.46 (1H, s, NHNHCSNH), 8.48 (1H, s, H^4), 8.26 (1H, d, H^8), 8.06 (1H, s, NHNHCSNH), 8.00-7.88 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.32-7.18 (5H, m, phenyl-H), 4.87 (2H, s, CH_2), 3.66-3.64 (2H, m, $\text{CH}_2\text{CH}_2\text{C}_6\text{H}_5$), 2.84 (2H, t, $\text{CH}_2\text{CH}_2\text{C}_6\text{H}_5$).

6d. IR (KBr) ν_{max} (cm^{-1}) 1674, 1645; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 10.47 (1H, s, CONH), 9.93 (1H, s, NHNHCSNH), 9.59 (1H, s, NHNHCSNH), 8.50 (1H, s, H^4), 8.26 (1H, d, H^8), 8.00-7.89 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.58 (2H, d, phenyl- $\text{H}^{2,6}$), 7.43 (2H, t, phenyl- $\text{H}^{3,5}$), 4.93 (2H, s, CH_2).

6e. IR (KBr) ν_{max} (cm^{-1}) 1685, 1648; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 10.38 (1H, s, CONH), 9.68 (1H, s, NHNHCSNH), 9.46 (1H, s, NHNHCSNH), 8.48 (1H, s, H^4), 8.24 (1H, d, H^8), 7.99-7.88 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.36 (2H, d, phenyl- $\text{H}^{2,6}$), 6.93 (2H, t, phenyl- $\text{H}^{3,5}$), 4.92 (2H, s, CH_2), 3.76 (3H, s, OCH_3).

6f. IR (KBr) ν_{max} (cm^{-1}) 1675, 1647; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 10.42 (1H, s,

CONH), 9.75 (1H, s, NHNHCSNH), 9.48 (1H, s, NHNHCSNH), 8.49 (1H, s, H^4), 8.24 (1H, d, H^8), 8.01-7.89 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.38 (2H, d, phenyl- $\text{H}^{2,6}$), 7.16 (2H, t, phenyl- $\text{H}^{3,5}$), 4.92 (2H, s, CH_2), 2.30 (3H, s, CH_3).

6g. IR (KBr) ν_{max} (cm^{-1}) 1701, 1632; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 10.03 (1H, s, CONH), 9.56 (2H, s, NHNHCSNH), 8.41 (1H, s, H^4), 8.16 (1H, d, H^8), 7.93-7.81 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.42 (2H, d, phenyl- $\text{H}^{2,6}$), 7.32 (2H, t, phenyl- $\text{H}^{3,5}$), 7.16 (1H, t, phenyl- H^4), 4.39 (2H, t, NCH_2), 2.68 (2H, t, CH_2CO).

6h. IR (KBr) ν_{max} (cm^{-1}) 1706, 1637; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 9.93 (1H, s, CONH), 9.34 (1H, s, NHNHCSNH), 8.43 (1H, s, NHNHCSNH), 8.41 (1H, s, H^4), 8.15 (1H, d, H^8), 7.93-7.81 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.30-7.21 (5H, m, phenyl-H), 4.77 (2H, d, $\text{CH}_2\text{C}_6\text{H}_5$), 4.39 (2H, t, NCH_2), 2.65 (2H, t, CH_2CO).

6i. IR (KBr) ν_{max} (cm^{-1}) 1699, 1641; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 9.83 (1H, s, CONH), 9.21 (1H, s, NHNHCSNH), 8.43 (1H, s, H^4), 8.24 (1H, d, H^8), 7.97 (1H, s, NHNHCSNH), 7.94-7.83 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.29-7.15 (5H, m, phenyl-H), 4.37 (2H, t, NCH_2), 3.64-3.60 (2H, m, $\text{CH}_2\text{CH}_2\text{C}_6\text{H}_5$), 2.79 (2H, t, $\text{CH}_2\text{CH}_2\text{C}_6\text{H}_5$), 2.63 (2H, t, CH_2CO).

6j. IR (KBr) ν_{max} (cm^{-1}) 1672, 1649; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 10.07 (1H, s, CONH), 9.69 (1H, s, NHNHCSNH), 9.61 (1H, s, NHNHCSNH), 8.45 (1H, s, H^4), 8.20 (1H, d, H^8), 7.97-7.84 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.49 (2H, d, phenyl- $\text{H}^{2,6}$), 7.40 (2H, t, phenyl- $\text{H}^{3,5}$), 4.42 (2H, t, NCH_2), 2.70 (2H, t, CH_2CO).

6k. IR (KBr) ν_{max} (cm^{-1}) 1682, 1665; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 10.01 (1H, s, CONH), 9.47 (1H, s, NHNHCSNH), 9.44 (1H, s, NHNHCSNH), 8.43 (1H, s, H^4), 8.19 (1H, d, H^8), 7.95-7.83 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.27 (2H, d, phenyl- $\text{H}^{2,6}$), 6.91 (2H, t, phenyl- $\text{H}^{3,5}$), 4.41 (2H, t, NCH_2), 3.76 (3H, s, OCH_3), 2.69 (2H, t, CH_2CO).

6l. IR (KBr) ν_{max} (cm^{-1}) 1703, 1633; $^1\text{H-NMR}$ (400 MHz) (DMSO-d_6) δ (ppm) 9.99 (1H, s, CONH), 9.74 (1H, s, NHNHCSNH), 9.48 (1H, s, NHNHCSNH), 8.41 (1H, s, H^4), 8.17 (1H, d, H^8), 7.92-7.82 (3H, m, $\text{H}^5, \text{H}^6, \text{H}^7$), 7.36 (2H, d, phenyl- $\text{H}^{2,6}$), 7.10 (2H, t, phenyl- $\text{H}^{3,5}$), 4.39 (2H, t, NCH_2), 2.67 (2H, t, CH_2CO), 2.25 (3H, s, CH_3).

General procedure for 3-[(1(2H)-phthalazinone-2-yl)(methyl)-4-aryl-1,2,4-triazole-5-thiones (7a-f)]. To a solution of the [1(2H)-phthalazinone-2-yl]acetylthiosemicarbazide (0.001 mol) in 50 mL methanol was added an aqueous solution of Na_2CO_3 (10 mL; 10%) and the

reaction mixture was refluxed for 4 h, cooled and then neutralized with glacial acetic acid (10%). The precipitate formed was filtered, washed with water and crystallized from an appropriate solvent.

7a. (KBr) ν_{\max} (cm^{-1}) 1650; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.97 (1H, s, NH), 8.34 (1H, s, H^4), 8.06 (1H, d, H^8), 7.91-7.80 (3H, m, $\text{H}^{5,6,7}$), 7.32-7.29 (5H, m, phenyl-H), 5.26 (2H, s, CH_2). Analysis for $\text{C}_{17}\text{H}_{13}\text{N}_5\text{OS}$ (335.38); Calcd: C; 60.88, H; 3.91, N; 20.88. Found: C; 60.89, H; 3.99, N; 20.71%.

7b. IR (KBr) ν_{\max} (cm^{-1}) 1630; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.97 (1H, s, NH), 8.24 (1H, s, H^4), 8.08 (1H, d, H^8), 7.86-7.76 (3H, m, $\text{H}^{5,6,7}$), 6.99-6.85 (5H, m, phenyl-H), 5.36 (2H, s, CH_2), 5.24 (2H, s, $\text{CH}_2\text{-C}_6\text{H}_5$). Analysis for $\text{C}_{18}\text{H}_{15}\text{N}_5\text{OS}$ (349.41); Calcd: C; 61.87, H; 4.33, N; 20.04. Found: C; 61.92, H; 4.33, N; 19.72%.

7c. IR (KBr) ν_{\max} (cm^{-1}) 1652; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.97 (1H, s, NH), 8.49 (1H, s, H^4), 8.28 (1H, d, H^8), 7.98-7.89 (3H, m, $\text{H}^{5,6,7}$), 7.31-7.23 (3H, m, phenyl- $\text{H}^{3,4,5}$), 7.13 (2H, d, phenyl- $\text{H}^{2,6}$), 5.19 (2H, s, CH_2), 4.18 (2H, t, $\text{N-CH}_2\text{-CH}_2\text{-C}_6\text{H}_5$), 2.85 (2H, t, $\text{N-CH}_2\text{-CH}_2\text{-C}_6\text{H}_5$). Analysis for $\text{C}_{19}\text{H}_{17}\text{N}_5\text{OS}$ (363.43); Calcd: C; 62.79, H; 4.71, N; 19.27. Found: C; 62.36, H; 4.64, N; 18.97%.

7d. IR (KBr) ν_{\max} (cm^{-1}) 1634; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.97 (1H, s, NH), 8.34 (1H, s, H^4), 8.05 (1H, d, H^8), 7.92-7.82 (3H, m, $\text{H}^{5,6,7}$), 7.33 (4H, s, phenyl-H), 5.30 (2H, s, CH_2). Analysis for $\text{C}_{17}\text{H}_{12}\text{ClN}_5\text{OS}$ (369.83); Calcd: C; 55.21, H; 3.27, N; 18.94. Found: C; 55.28, H; 3.37, N; 18.87%.

7e. IR (KBr) ν_{\max} (cm^{-1}) 1647; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.86 (1H, s, NH), 8.35 (1H, s, H^4), 8.06 (1H, d, H^8), 7.92-7.81 (3H, m, $\text{H}^{5,6,7}$), 7.18 (2H, d, phenyl- $\text{H}^{3,5}$), 6.80 (2H, d, phenyl- $\text{H}^{2,6}$), 5.25 (2H, s, CH_2), 3.64 (3H, s, OCH_3). Analysis for $\text{C}_{18}\text{H}_{15}\text{N}_5\text{O}_2\text{S}$ (365.41); Calcd: C; 59.16, H; 4.14, N; 19.17. Found: C; 58.85, H; 4.05, N; 18.80%.

7f. IR (KBr) ν_{\max} (cm^{-1}) 1665; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.87 (1H, s, NH), 8.34 (1H, s, H^4), 8.05 (1H, d, H^8), 7.93-7.80 (3H, m, $\text{H}^{5,6,7}$), 7.14 (2H, d, phenyl- $\text{H}^{3,5}$), 7.07 (2H, d, phenyl- $\text{H}^{2,6}$), 5.25 (2H, s, CH_2), 2.16 (3H, s, CH_3). Analysis for $\text{C}_{18}\text{H}_{15}\text{N}_5\text{OS}$ (349.41); Calcd: C; 61.87, H; 4.33, N; 20.04. Found: C; 62.00, H; 4.32, N; 19.95%.

General procedure for 3-[(1(2H)-phthalazinone-2-yl(ethyl)-4-aryl-1,2,4-triazole-5-thione) (7g-l)]. A NaOH pellet (0.01 mol) was added a solution of the [1(2H)-phthalazinone-2-yl]propanoylthiosemicarbazide (0.001 mol) in 50 mL ethanol and the mixture

was refluxed until the starting material had been consumed. At the end of this period, the resulting solution was poured into ice water and neutralized with glacial acetic acid (10%). The precipitate formed was filtered, washed with water and crystallized from an appropriate solvent.

7g. IR (KBr) ν_{\max} (cm^{-1}) 1635; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.65 (1H, s, NH), 8.39 (1H, s, H^4), 8.19 (1H, d, H^8), 7.94-7.85 (3H, m, $\text{H}^{5,6,7}$), 7.52-7.41 (5H, m, phenyl-H), 4.26 (2H, t, phthalazinone- CH_2CH_2), 2.29 (2H, t, phthalazinone- CH_2CH_2). Analysis for $\text{C}_{18}\text{H}_{15}\text{N}_5\text{OS}$ (349.41); Calcd: C; 61.87, H; 4.33, N; 20.04. Found: C; 61.83, H; 4.13, N; 20.22%.

7h. IR (KBr) ν_{\max} (cm^{-1}) 1626; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.68 (1H, s, NH), 8.39 (1H, s, H^4), 8.22 (1H, d, H^8), 7.94-7.85 (3H, m, $\text{H}^{5,6,7}$), 7.34-7.26 (5H, m, phenyl-H), 5.30 (2H, s, $\text{CH}_2\text{-C}_6\text{H}_5$), 4.36 (2H, t, phthalazinone- CH_2CH_2), 3.04 (2H, t, phthalazinone- CH_2CH_2). Analysis for $\text{C}_{19}\text{H}_{17}\text{N}_5\text{OS}$ (363.43); Calcd: C; 62.79, H; 4.41, N; 19.27. Found: C; 62.52, H; 4.71, N; 19.23%.

7i. IR (KBr) ν_{\max} (cm^{-1}) 1653; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.53 (1H, s, NH), 8.44 (1H, s, H^4), 8.25 (1H, d, H^8), 7.95-7.85 (3H, m, $\text{H}^{5,6,7}$), 7.31-7.20 (5H, m, phenyl-H), 4.36 (2H, t, phthalazinone- CH_2CH_2), 4.14 (2H, t, $\text{N-CH}_2\text{CH}_2\text{C}_6\text{H}_5$), 3.02 (2H, t, $\text{N-CH}_2\text{CH}_2\text{C}_6\text{H}_5$), 2.87 (2H, t, phthalazinone- CH_2CH_2). Analysis for $\text{C}_{20}\text{H}_{19}\text{N}_5\text{OS}$ (377.46); Calcd: C; 63.64, H; 5.07, N; 18.55. Found: C; 63.28, H; 5.07, N; 18.48%.

7j. IR (KBr) ν_{\max} (cm^{-1}) 1634; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.70 (1H, s, NH), 8.39 (1H, s, H^4), 8.19 (1H, d, H^8), 7.94-7.84 (3H, m, $\text{H}^{5,6,7}$), 7.57 (2H, d, phenyl- $\text{H}^{3,5}$), 7.50 (2H, d, phenyl- $\text{H}^{2,6}$), 4.26 (2H, t, phthalazinone- CH_2CH_2), 2.98 (2H, t, phthalazinone- CH_2CH_2). Analysis for $\text{C}_{18}\text{H}_{14}\text{ClN}_5\text{OS}$ (383.85); Calcd: C; 56.32, H; 3.68, N; 18.24. Found: C; 56.24, H; 3.69, N; 18.12%.

7k. IR (KBr) ν_{\max} (cm^{-1}) 1633; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.60 (1H, s, NH), 8.40 (1H, s, H^4), 8.19 (1H, d, H^8), 7.94-7.86 (3H, m, $\text{H}^{5,6,7}$), 7.33 (2H, d, phenyl- $\text{H}^{3,5}$), 7.01 (2H, d, phenyl- $\text{H}^{2,6}$), 4.26 (2H, t, phthalazinone- CH_2CH_2), 3.35 (3H, s, OCH_3), 2.94 (2H, t, phthalazinone- CH_2CH_2). Analysis for $\text{C}_{19}\text{H}_{17}\text{N}_5\text{OS}$ (363.43); Calcd: C; 62.79, H; 4.71, N; 19.27. Found: C; 62.37, H; 4.79, N; 19.18%.

7l. IR (KBr) ν_{\max} (cm^{-1}) 1634; $^1\text{H-NMR}$ (400 MHz) (DMSO- d_6) δ (ppm) 13.62 (1H, s, NH), 8.39 (1H, s, H^4), 8.19 (1H, d, H^8), 7.94-7.84 (3H, m, $\text{H}^{5,6,7}$), 7.27 (4H, s, phenyl-H), 4.26 (2H, t, phthalazinone- CH_2CH_2), 2.95 (2H, t, phthalazinone- CH_2CH_2), 2.34 (3H, s, CH_3). Analysis for $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_2\text{S}$ (379.73); Calcd: C; 60.14, H; 4.52, N; 18.46. Found: C; 60.01, H; 4.55, N; 18.46%.

General procedure for 2-[(phthalazinone-2-yl(methyl)-5-arylamino-1,3,4-thiadiazoles (**8a-f**). The appropriate [1(2H)-phthalazinone-2-yl]acetylthiosemicarbazide derivative (0.001 mol) was added portion-wise to H₂SO₄ (5 mL) cooled in an ice bath with constant stirring. After dissolution, the reaction mixture was further stirred for 30 min, poured over crushed ice and neutralized by saturated Na₂CO₃ solution at room temperature. Then the solid material was filtered, washed with water and crystallized from an appropriate solvent.

8a. IR (KBr) ν_{\max} (cm⁻¹) 1645; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 10.32 (1H, s, NH), 8.51 (1H, s, H⁴), 8.30 (1H, d, H⁸), 7.98-7.88 (3H, m, H^{5,6,7}), 7.55 (2H, d, phenyl-H^{2,6}), 7.31 (2H, t, phenyl-H^{3,5}), 6.97 (1H, t, phenyl-H⁴), 5.55 (2H, s, CH₂). Analysis for C₁₇H₁₃N₅OS (335.38); Calcd: C; 60.88, H; 3.91, N; 20.88. Found: C; 60.85, H; 3.64, N; 21.00%.

8b. IR (KBr) ν_{\max} (cm⁻¹) 1648; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 8.46 (1H, s, H⁴), 8.25 (1H, d, H⁸), 8.21 (1H, t, NH), 7.94-7.85 (3H, m, H^{5,6,7}), 7.30-7.21 (5H, m, phenyl-H), 5.43 (2H, s, CH₂), 4.41 (2H, d, NH-CH₂C₆H₅). Analysis for C₁₈H₁₅N₅OS (349.41); Calcd: C; 61.87, H; 4.33, N; 20.04. Found: C; 62.08, H; 4.36, N; 20.10%.

8c. IR (KBr) ν_{\max} (cm⁻¹) 1668; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 8.51 (1H, s, H⁴), 8.29 (1H, d, H⁸), 7.98-7.90 (3H, m, H^{5,6,7}), 7.82 (1H, t, NH), 7.29-7.17 (5H, m, phenyl-H), 5.46 (2H, s, CH₂), 3.46 (2H, q, NH-CH₂CH₂C₆H₅), 2.84 (2H, t, NH-CH₂CH₂C₆H₅). Analysis for C₁₉H₁₇N₅OS (363.43); Calcd: C; 62.79, H; 4.71, N; 19.27. Found: C; 62.20, H; 4.68, N; 19.05%.

8d. IR (KBr) ν_{\max} (cm⁻¹) 1643; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 10.46 (1H, s, NH), 8.51 (1H, s, H⁴), 8.29 (1H, d, H⁸), 7.98-7.88 (3H, m, H^{5,6,7}), 7.60 (2H, d, phenyl-H^{2,6}), 7.36 (2H, t, phenyl-H^{3,5}), 5.56 (2H, s, CH₂). Analysis for C₁₇H₁₂ClN₅OS (369.82); Calcd: C; 55.21, H; 3.27, N; 18.94. Found: C; 54.82, H; 3.20, N; 18.69%.

8e. IR (KBr) ν_{\max} (cm⁻¹) 1645; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 10.10 (1H, s, NH), 8.51 (1H, s, H⁴), 8.28 (1H, d, H⁸), 7.97-7.88 (3H, m, H^{5,6,7}), 7.45 (2H, d, phenyl-H^{2,6}), 6.89 (2H, d, phenyl-H^{3,5}), 5.52 (2H, s, CH₂), 3.70 (3H, s, OCH₃). Analysis for C₁₈H₁₅N₅OS (349.41); Calcd: C; 61.87, H; 4.33, N; 20.04. Found: C; 61.39, H; 4.32, N; 19.75%.

8f. IR (KBr) ν_{\max} (cm⁻¹) 1636; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 10.20 (1H, s, NH), 8.51 (1H, s, H⁴), 8.28 (1H, d, H⁸), 7.98-7.89 (3H, m, H^{5,6,7}), 7.42 (2H, d, phenyl-H^{2,6}), 7.11 (2H, d, phenyl-H^{3,5}), 5.54 (2H, s, CH₂), 2.22 (3H, s, CH₃). Analysis for C₁₈H₁₅N₅O₂S (365.41); Calcd: C; 59.16, H; 4.14, N; 19.17. Found: C; 59.12, H; 4.10, N; 18.99%.

General procedure for 2-[(phthalazinone-2-yl(ethyl)-5-arylamino-1,3,4-thiadiazoles (**8g-l**). [1(2H)-phthalazinone-2-yl]propanoylthiosemicarbazides (0.001 mol) (**6**) were dissolved in 10 mL toluene and *p*-toluenesulfonic acid (0.0015 mol) was added and refluxed until the starting material had been consumed. At the end of this period, the reaction mixture was neutralized with 10% ammonium hydroxide. Then the solid material was filtered, washed with water and crystallized from an appropriate solvent.

8g. IR (KBr) ν_{\max} (cm⁻¹) 1651; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 10.22 (1H, s, NH), 8.46 (1H, s, H⁴), 8.26 (1H, d, H⁸), 7.96-7.87 (3H, m, H^{5,6,7}), 7.54 (2H, d, phenyl-H^{2,6}), 7.30 (2H, t, phenyl-H^{3,5}), 6.96 (1H, t, phenyl-H⁴), 4.49 (2H, t, phthalazinone-CH₂CH₂), 3.44 (2H, t, phthalazinone-CH₂CH₂). Analysis for C₁₈H₁₅N₅OS (349.41); Calcd: C; 61.87, H; 4.33, N; 20.04. Found: C; 62.09, H; 4.24, N; 19.99%.

8h. IR (KBr) ν_{\max} (cm⁻¹) 1645; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 8.44 (1H, s, H⁴), 8.25 (1H, d, H⁸), 8.07 (1H, t, NH), 7.96-7.86 (3H, m, H^{5,6,7}), 7.34-7.25 (5H, m, phenyl-H), 4.43-4.40 (4H, m, phthalazinone-CH₂CH₂, NH-CH₂C₆H₅), 3.32 (2H, t, phthalazinone-CH₂CH₂). Analysis for C₁₉H₁₇N₅OS (363.43); Calcd: C; 62.79, H; 4.71, N; 19.27. Found: C; 63.12, H; 4.75, N; 19.23%.

8i. IR (KBr) ν_{\max} (cm⁻¹) 1647; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 8.46 (1H, s, H⁴), 8.26 (1H, d, H⁸), 7.96-7.86 (3H, m, H^{5,6,7}), 7.67 (1H, t, NH), 7.30-7.18 (5H, m, phenyl-H), 4.43 (2H, t, phthalazinone-CH₂CH₂), 3.42 (2H, q, NH-CH₂CH₂C₆H₅), 3.33 (2H, t, phthalazinone-CH₂CH₂), 2.83 (2H, t, NH-CH₂CH₂C₆H₅). Analysis for C₂₀H₁₉N₅OS (377.46); Calcd: C; 63.64, H; 5.07, N; 18.55. Found: C; 63.59, H; 5.18, N; 18.51%.

8j. IR (KBr) ν_{\max} (cm⁻¹) 1662; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 10.37 (1H, s, NH), 8.46 (1H, s, H⁴), 8.22 (1H, d, H⁸), 7.96-7.88 (3H, m, H^{5,6,7}), 7.59 (2H, d, phenyl-H^{2,6}), 7.35 (2H, d, phenyl-H^{3,5}), 4.49 (2H, t, phthalazinone-CH₂CH₂), 3.44 (2H, t, phthalazinone-CH₂CH₂). Analysis for C₁₈H₁₄ClN₅OS (383.85); Calcd: C; 56.32, H; 3.68, N; 18.24. Found: C; 55.45, H; 3.53, N; 17.92%.

8k. IR (KBr) ν_{\max} (cm⁻¹) 1652; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 9.99 (1H, s, NH), 8.44 (1H, s, H⁴), 8.24 (1H, d, H⁸), 7.94-7.86 (3H, m, H^{5,6,7}), 7.44 (2H, d, phenyl-H^{2,6}), 6.87 (2H, d, phenyl-H^{3,5}), 4.46 (2H, t, phthalazinone-CH₂CH₂), 3.70 (3H, s, OCH₃), 3.39 (2H, t, phthalazinone-CH₂CH₂). Analysis for C₁₉H₁₇N₅OS (363.43); Calcd: C; 62.79, H; 4.71, N; 19.27. Found: C; 61.91, H; 4.65, N; 18.86%.

8l. IR (KBr) ν_{\max} (cm⁻¹) 1646; ¹H-NMR (400 MHz) (DMSO-d₆) δ (ppm) 10.10 (1H, s, NH), 8.46 (1H, s,

Table I. Physicochemical data of [1(2H)-Phthalazinone-2-yl]acetyl/propanoylthiosemicarbazide derivatives (6a–1).

Comp.	n	R	(Cryst. Sol)	mp	Yield (%)
6a	1	Phenyl	Ethanol	207–209	75
6b	1	Benzyl	Methanol	208	55
6c	1	Phenethyl	Methanol	225	50
6d	1	4-Chlorophenyl	Methanol	237	65
6e	1	4-Methoxyphenyl	Methanol	223–224	60
6f	1	4-Methylphenyl	Methanol	210	65
6g	2	Phenyl	Methanol	205	60
6h	2	Benzyl	Methanol	209,5	45
6i	2	Phenethyl	Ethanol	196–197	40
6j	2	4-Chlorophenyl	Ethanol	186	55
6k	2	4-Methoxyphenyl	Ethanol	201	50
6l	2	4-Methylphenyl	Ethanol	208	55

H⁴), 8.26 (1H, d, H⁸), 7.96–7.85 (3H, m, H^{5,6,7}), 7.42 (2H, d, phenyl-H^{2,6}), 7.10 (2H, d, phenyl-H^{3,5}), 4.84 (2H, t, phthalazinone-CH₂CH₂), 3.44 (2H, t, phthalazinone-CH₂CH₂), 2.24 (3H, s, CH₃). Analysis for C₁₉H₁₇N₅O₂S (379.43); Calcd: C; 60.14, H; 4.52, N; 18.46. Found: C; 60.01, H; 4.55, N; 18.46%.

Antimicrobial activity

Minimum inhibitory concentration (MIC) values for the synthesized compounds were determined by using the broth microdilution method [13,14]. Two Gram-positive (*S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633) and two Gram-negative (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) bacteria were used as quality control strains. For determining anti-yeast activities of the compounds, the following reference strains were tested: *Candida albicans* ATCC 10231 and

Candida parapsilosis ATCC 90018. Ampicillin trihydrate and fluconazole were used as standard antibacterial and antifungal agents, respectively. Fluconazole was dissolved in sterile distilled water, ampicillin trihydrate in phosphate buffer (pH 8) and the stock solution of the synthesized compounds was dissolved in dimethyl sulfoxide (DMSO) and distilled water (50%) at a concentration of 2048 µg/mL. Twofold dilutions of the synthesized compounds were prepared (1024, 512, 2 µg/mL), and twofold dilutions of the reference compounds were prepared at 64–0.125 µg/mL. All bacteria were cultivated in Mueller–Hinton Agar (Merck). The bacteria inoculums was prepared in Mueller–Hinton Broth (Merck) which had been kept at 36°C overnight and was diluted with broth to give a final concentration of 5 × 10⁵ cfu/mL. All fungi were cultivated in Sabouraud Dextrose Agar (Merck). The fungi inoculums

Table II. Physicochemical data for the title compounds (7a–1, 8a–1).

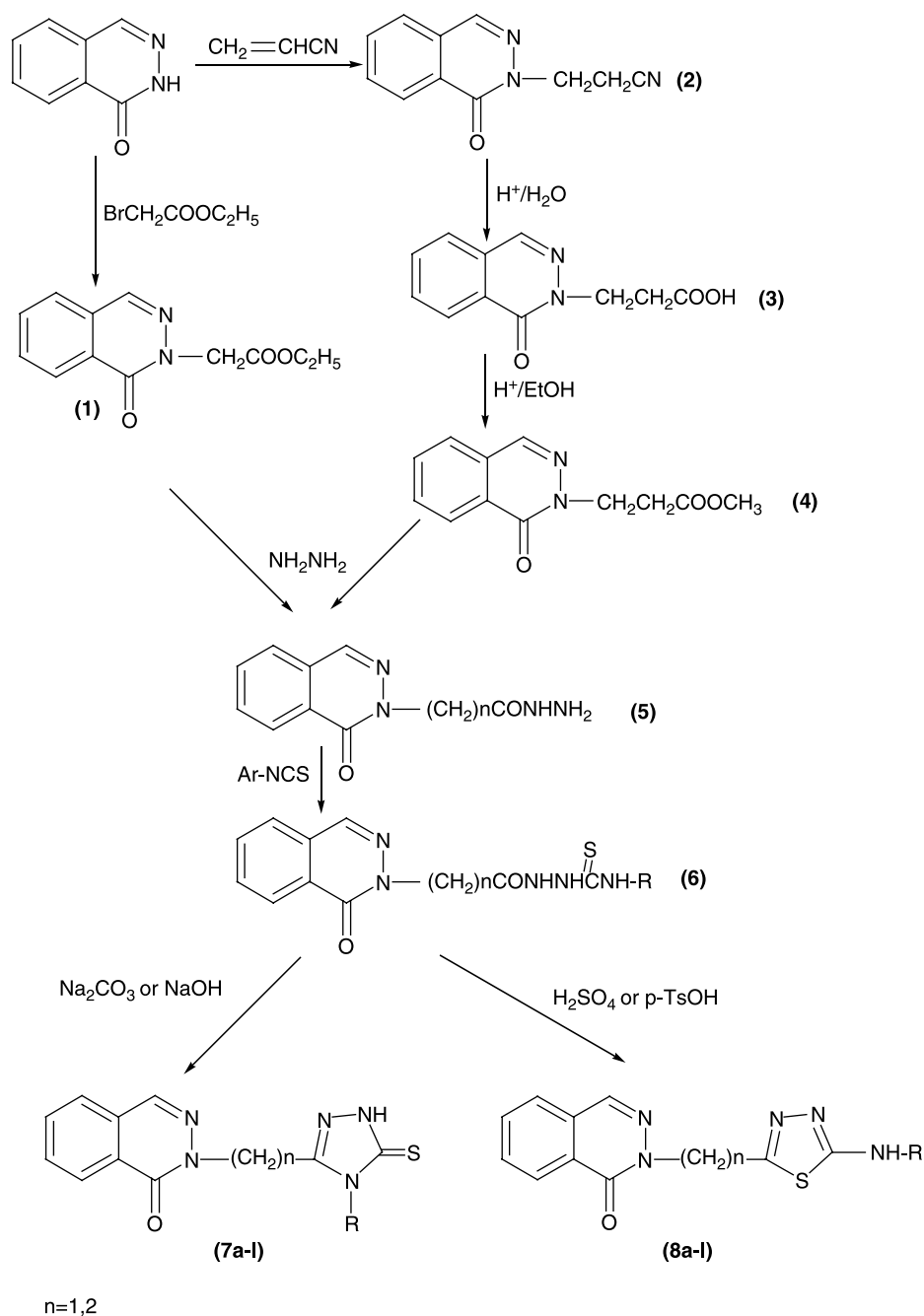
Comp.	n	R	Cryst. Sol	Yield (%)	Mp (°C)
7a	1	Phenyl	Methanol	65	238–240
7b	1	Benzyl	Ethanol	45	230–231
7c	1	Phenethyl	Methanol	40	231–233
7d	1	4-Chlorophenyl	Ethanol	50	239–240
7e	1	4-Methoxyphenyl	2-Propanol	45	210–211
7f	1	4-Methylphenyl	Ethanol	55	> 300
7g	2	Phenyl	Ethanol-Water	60	168–170
7h	2	Benzyl	Ethanol	40	193–194
7i	2	Phenethyl	Methanol	42	216–217
7j	2	4-Chlorophenyl	Methanol	50	228–229
7k	2	4-Methoxyphenyl	Ethanol	55	225
7l	2	4-Methylphenyl	Methanol	55	239–241
8a	1	Phenyl	Ethanol	60	228–231
8b	1	Benzyl	Ethanol	55	170–171
8c	1	Phenethyl	Methanol	50	175–176
8d	1	4-Chlorophenyl	Ethanol-Water	45	273
8e	1	4-Methoxyphenyl	Ethanol-Water	50	207
8f	1	4-Methylphenyl	Ethanol	55	223
8g	2	Phenyl	Ethanol	60	202–203
8h	2	Benzyl	Acetone-P.Ether	55	165–166
8i	2	Phenethyl	Ethanol	45	161
8j	2	4-Chlorophenyl	Toluene	50	225–226
8k	2	4-Methoxyphenyl	Ethanol	55	183–184
8l	2	4-Methylphenyl	Methanol	55	184–185

were prepared in Sabouraud liquid medium (Oxoid) which had been kept at 36°C overnight and was diluted with RPMI-1640 medium with L-glutamine buffered with 3-[N-morpholino]-propanesulfonic acid (MOPS) at pH 7 to give a final concentration of 2.5×10^3 cfu/mL. The microplates were incubated at 36°C and read visually after 24 h, except for *Candida* species when it was at 48 h. The incubation chamber was kept humid. At the end of the incubation period, MIC values were recorded as the lowest concentrations of the substances that gave no visible turbidity. The DMSO diluents at a maximum final concentration of 12.5% had no effect on the microorganism's growth.

Results and discussion

Chemistry

1(2H)-Phthalazinone was used as the starting material. Synthesis of the compounds 1–3 and [1(2H)-phthalazinone-2-yl]acetylhydrazine were accomplished according to previously reported procedures [12,15]. Methyl [1(2H)-phthalazinone-2-yl]propanoate (SciFinder Scholar 2006, 618441-99-9) and 3-[1(2H)-phthalazinone-2-yl]propanoylhydrazine (618442-00-5) were synthesized according to the available procedures. Synthesis of the novel compounds 6–8 is reported in this study. Structures of the



Scheme 1. Synthetic route for the title compounds.

Table III. Minimum inhibitory concentrations (MICs, $\mu\text{g/mL}$) for the title compounds.

Compounds	<i>Staphylococcus aureus</i> ATCC 25923	<i>Bacillus subtilis</i> ATCC 6633	<i>Escherichia coli</i> ATCC 25922	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Candida albicans</i> ATCC 10231	<i>Candida parapsilosis</i> ATCC 90018
7a	512	512	256	256	512	512
7b	512	32	256	256	128	64
7c	256	256	128	256	32	32
7d	512	64	256	256	512	512
7e	512	32	256	256	512	512
7f	512	64	256	256	512	512
7g	512	64	256	256	512	256
7h	512	64	256	256	64	64
7i	512	32	256	256	256	32
7j	512	64	256	256	512	512
7k	512	64	256	256	64	32
7l	512	64	256	256	512	512
8a	512	16	128	256	512	64
8b	128	32	128	256	128	64
8c	256	64	128	256	32	32
8d	256	256	128	512	32	32
8e	256	256	128	512	32	32
8f	512	256	128	512	128	32
8g	128	32	256	256	128	16
8h	256	128	256	256	512	128
8i	512	32	256	256	32	64
8j	512	128	256	512	128	64
8k	512	32	256	256	128	64
8l	512	32	256	256	128	16
Ampicillin	4	8	4	–	–	–
Fluconazole	–	–	–	–	8	0.25

title compounds were confirmed by IR, $^1\text{H-NMR}$ and elemental analyses. [1(2H)-Phthalazinone-2-yl]acetyl/propanoylthiosemicarbazide derivatives (**6a–1**) were prepared by the reaction of [1(2H)-phthalazinone-2-yl]acetyl/propanoylhydrazine with the appropriate isothiocyanate derivative in methanol or ethanol. [1(2H)-Phthalazinone-2-yl]acetyl/propanoylthiosemicarbazide derivatives (**6a–1**) were cyclized to the corresponding 3-[(1(2H)-phthalazinone-2-yl(methyl/ethyl)-4-aryl-1,2,4-triazole-5-thione derivatives (**7a–1**) under alkaline conditions. The other title compounds, 2-[(phthalazinone-2-yl(methyl/ethyl)-5-arylamino-1,3,4-thiadiazole derivatives (**8a–1**) were obtained by the cyclization of [1(2H)-phthalazinone-2-yl]-acetyl/propanoylthiosemicarbazide derivatives (**7a–1**) under acidic conditions.

In this study, we selected aromatic isothiocyanate derivatives to synthesize the acyl thiosemicarbazide derivatives, since the acyl thiosemicarbazides carrying an aromatic substituents gave good yield when cyclized to 1,2,4-triazole and 1,3,4-thiadiazole derivatives. In addition, we preferred to synthesize 1,2,4-triazole and 1,3,4-thiadiazole derivatives which were attached at position-2 of the phthalazinone ring through methylene or ethylene bridges, since many compounds that carry 1,2,4-triazole and 1,3,4-thiadiazole rings have been reported to be attached to other rings through methylene or ethylene bridges in the literature [5,16].

Physicochemical data for the [1(2H)-Phthalazinone-2-yl]acetyl/propanoylthiosemicarbazide derivatives

(**6a–1**) is given in Table I whereas that for (**7a–1**) and (**8a–1**) is given in Table II. The synthetic route for the title compounds is shown in Scheme 1.

Antimicrobial activity

The synthesized compounds were tested against two Gram (+) bacteria (*S. aureus*, *B. subtilis*), two Gram (–) bacteria (*P. aeruginosa*, *E. coli*) and two yeast-like fungi *C. albicans* and *C. parapsilosis* using the broth micro-dilution method. Ampicillin and fluconazole were used as standard antibacterial and standard antifungal agents, respectively.

As shown in Table III, none of the title compounds had activity against *S. aureus*, *P. aeruginosa* and *E. coli* but, generally, the title compounds were found to be active against *B. subtilis* and the fungi. Derivatives carrying 1,3,4-thiadiazole ring, generally, showed higher antimicrobial activity against *B. subtilis* and the fungi compared to the other compounds. The antibacterial activity of **8a** was 50% of that of ampicillin against *B. subtilis*. This derivative was found to be more active against *B. subtilis* when compared to the other synthesized compounds. The antibacterial activity of compounds **7b**, **7e**, **7i**, **8b**, **8g**, **8i**, **8k** and **8l** was 25% of that of ampicillin against *B. subtilis*. Therefore it can be suggested that these compounds show promise as antibacterials and worth further work on their derivatives. The antifungal activity

of compounds **7c**, **8c–8e** and **8i** was 25% of that of fluconazole against *C. albicans*. The MIC values of **7k** against *C. albicans* and *C. parapsilosis* were determined as 64 µg/mL and 32 µg/mL, respectively. Compound **7i** was active against *C. parapsilosis* and its MIC value was 32 µg/mL. Compounds **8g** and **8l** exhibited antifungal activity with a MIC value of 16 µg/mL against *C. parapsilosis* and these values for **8c–8f** against *C. parapsilosis* were 32 µg/mL. Therefore, compounds **7c**, **7i**, **7k**, **8c–8e**, **8g**, **8i** and **8l** could be good starting points for developing better antifungal agents against yeast-like fungi. Chain elongation between the two heterocyclic rings may be a good choice for further studies. Additionally, further substitution on the phthalazinone moiety should be studied.

Acknowledgements

This study was supported by a grant from the Research Foundation of Gazi University (EF 02/2006-01).

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