ELSEVIER

www.elsevier.com/locate/farmac

Il Farmaco 56 (2001) 953–958

IL FARMACO

Synthesis and structure elucidation of some new thioether derivatives of 1,2,4-triazoline-3-thiones and their antimicrobial activities

N.N. Gülerman [a], H.N. Doğan [a], S. Rollas [a,*], C. Johansson [b], C. Çelik [b]

[a] *Faculty of Pharmacy, Pharmaceutical Chemistry Department, Marmara University, Haydarpaşa, Istanbul 81010, Turkey*
[b] *Faculty of Medicine, Microbiology Department, Marmara University, Haydarpaşa, Istanbul 81010, Turkey*

Received 25 October 2000; accepted 1 June 2001

Abstract

5-(4-Pyridinyl)-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones and 5-(4-pyridinyl)-4-substituted-3-(benzoylmethyl)thio-4*H*-1,2,4-triazoles were synthesized. The structures of original nine compounds were confirmed by IR, ^{1}H NMR, mass spectral methods and elemental analysis. The antibacterial, antifungal and antimycobacterial activities, together with those of known intermediate 1,4-disubstituted thiosemicarbazides, were reported.

Keywords: 1,2,4-Triazole derivatives; Synthesis; Antibacterial, antifungal; Antimycobacterial activities

1. Introduction

Certain 1,4-disubstituted thiosemicarbazide, 1,2,4-triazoline-3-thione and 1,2,4-triazole-3-thiole derivatives are of interest due to their bioactivity, including antibacterial [1,2], antifungal [3,4] and antitubercular [5,6] properties. We reported here on the synthesis and structure elucidation of 5-(4-pyridinyl)-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones and 5-(4-pyridinyl)-4-substituted-3-(benzoylmethyl)thio-4*H*-1,2,4-triazoles. The microbiological tests were also applied to these compounds and their known intermediate 1-(isonicotinoyl)-4-substituted thiosemicarbazides.

2. Chemistry

For the synthesis of 1-isonicotinoyl-4-substituted thiosemicarbazides (**1a**–**h**), isoniazid was added to ethyl, allyl, phenyl, *p*-(bromo/chloro/fluoro)phenyl, benzyl and phenethyl isothiocyanates [3,7]. Isoniazid was selected as the starting compound because of its antitubercular activity [8]. 1,2,4-Triazoline-3-thione derivatives (**2a**–**h**) were obtained by the cyclization of the thiosemicarbazides in alkaline medium [9,10] and two compounds among them (**2f** and **2h**) are original. For the synthesis of 5-(4-pyridinyl)-4-substituted-3-(benzoylmethyl)thio-4*H*-1,2,4-triazoles (**3a**–**g**), these triazoles (**2a**–**g**) were reacted with phenacyl chloride in acetone containing potassium carbonate, thus seven original compounds were obtained (Scheme 1).

According to IR spectroscopic data of two original compounds (**2f**, **2h**) which have triazoline-3-thione structure, the observation of C=S stretching bands of 1239 and 1261 cm^{-1} and the absence of an absorption about in 2600–2550 cm^{-1} region cited for SH group have proved that these compounds were in the thionic form in the solid state [11,12]. NH stretching bands of **2f** and **2h** were observed at 3120 and 3500–3350 cm^{-1}, respectively, and these bands disappeared in the IR spectra of **3a**–**g**. The IR spectra of thioether derivatives (**3a**–**g**) showed C–S stretching bands in the 641–630 cm^{-1} region. The compounds (**3a**–**g**) showed characteristic C=O stretching bands in the 1704–1674 cm^{-1} region. All data were consistent with the proposed structures [11–13].

In the ^{1}H NMR spectra of **2f** and **2h**, NH peaks were observed as a singlet at 14.35 and 14.15 ppm, respectively, and so their thionic form was proved. Oppositely, NH signals were not observed in the ^{1}H NMR

* Corresponding author.
E-mail address: sevim@sevimrollas.com (S. Rollas).

0014-827X/01/$ - see front matter
PII: S0014-827X(01)01167-3

spectra of **3a**–**g**. The methylene protons appeared as a singlet at 4.81–5.03 ppm. Compound **3b** showed *cis* and *trans* separation of protons depending on the nature of the allyl substituent [14]. Furthermore the signals of protons which characterized aromatic moiety of **3a**–**g**, $-CH_2CH_3$ protons of **3a** and $-NCH_2$ protons of **3g** were observed in the expected field and they are consistent with those of IR and literature data [14–16].

The mass spectra of **3b**, **3f** and **3g** which were selected as prototype, showed molecular ion (M^+) peaks, but their relative intensities were very low. The mass fragmentation patterns of these compounds were shown in Scheme 2.

3. Experimental

3.1. Chemistry

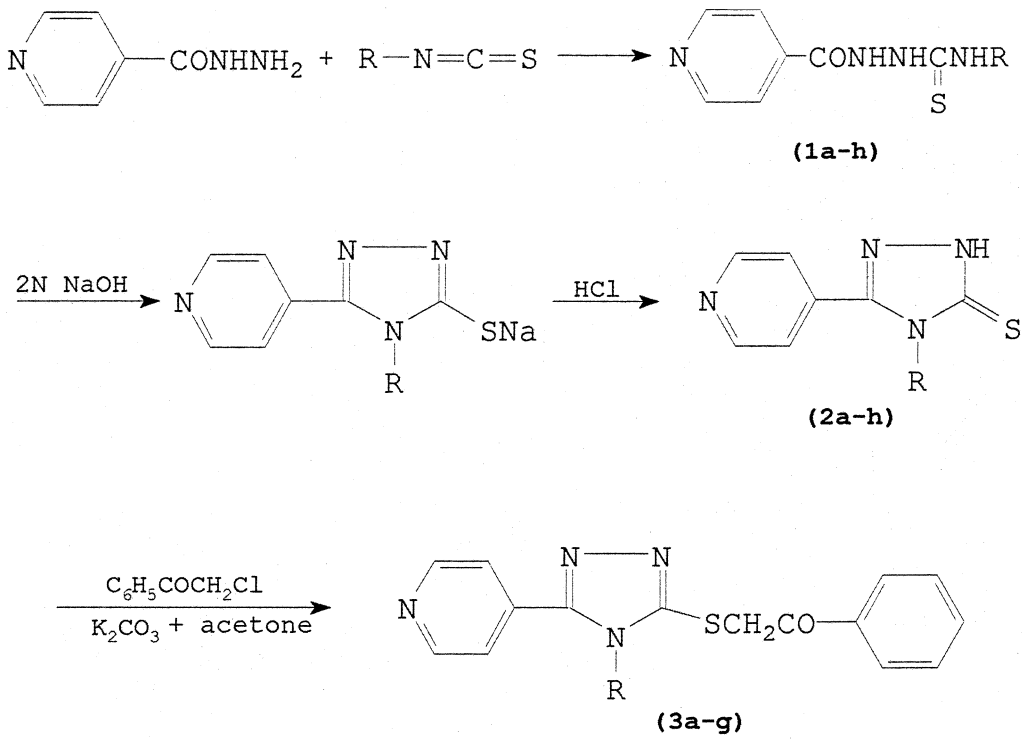
Chemicals used in the experiments were commercially available and were used without further purification.

All melting points were determined on a Buchi 530 melting point apparatus and uncorrected. The elemental analysis were performed on a Leco CHNS-932 instrument. IR spectra were obtained on a 1600 FTIR spectrophotometer as KBr pellets. ^{1}H NMR spectra were obtained on a Bruker AVANC-DPX 400 spectrometer in DMSO with TMS as internal reference. Mass spectra of **3b**, **3f** and **3g** were obtained on a Fisons Instruments VG Platform II LC-MS spectrometer.

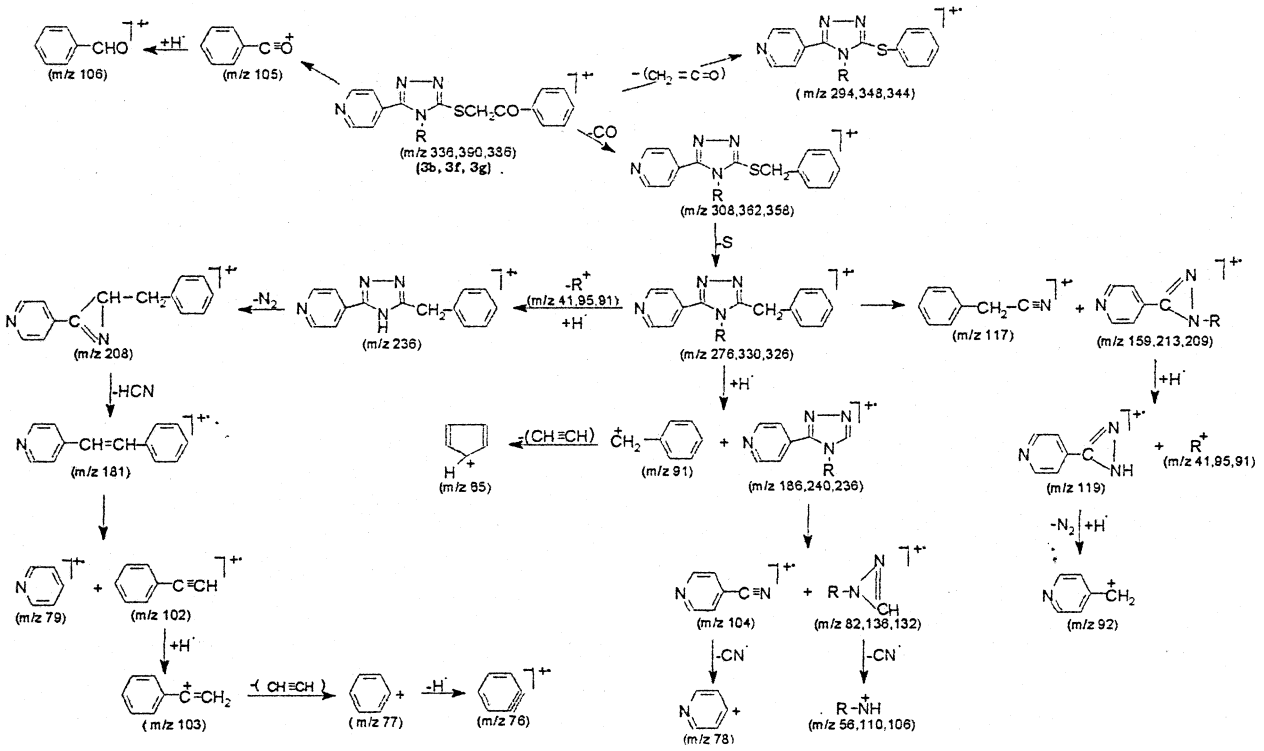
3.1.1. General procedure for the preparation of 5-(4-pyridinyl)-4-substituted-2,4-dihydro-3H-1,2,4-triazol-3-thiones (2a–h)

Equimolar amounts of isoniazid and substituted isothiocyanate were refluxed in ethanol for 3 h. The precipitate (**1a**–**h**) was recrystallized from ethanol [7] and then 0.0075 mol **1a**–**h** dissolved 2N sodium hydroxide (15 ml), heated under reflux for 4 h [9]. After cooling, the solution was acidified by hydrochloric acid, the crude product was precipitated, filtered and washed with distilled water. Pure compounds were obtained by crystallization from ethanol or methanol.

*3.1.1.1. 4-(4-Fluorophenyl)-5-(4-pyridinyl)-2,4-dihydro-3H-1,2,4-triazol-3-thione (**2f**).* $C_{13}H_9FN_4S$, m.wt. 272.3, yellow crystals, 91% yield, m.p. 260–261 °C (EtOH). Elemental analysis (%Calc./Found): 57.84/57.88 (C), 3.33/3.64 (H), 20.58/20.49 (N), 11.77/11.81% (S). IR (ν, cm^{-1}): 3120 (NH triazoline); 3045 (=CH aromatic); 1609, 1565, 1510, 1478, 1390 (C=C and C=N of rings); 1285 (C–N triazoline); 1239 (C=S); 1165 (Ar–F); 830 (1,4-disubstituted benzene and 4-substituted pyridine).



Scheme 1. Synthetic route to compounds.



Scheme 2. Mass fragmentations of **3b**, **3f** and **3g**.

^{1}H NMR, (δ, ppm): 7.26 (2H, dd, $J = 6.14$ Hz, C_3H and C_5H of pyridine), 7.38 (2H, t, $J = 8.77$ Hz, C_3H and C_5H of phenyl), 7.48–7.52 (2H, m, C_2H and C_6H of phenyl), 8.60 (2H, dd, $J = 6.02$ Hz, C_2H and C_6H of pyridine), 14.35 (1H, s, NH).

*3.1.1.2. 4-(Phenethyl)-5-(4-pyridinyl)-2,4-dihydro-3H-1,2,4-triazol-3-thione (**2h**).* $C_{15}H_{14}N_4S$, m.wt. 282.4, colourless cubic crystals, 60% yield, m.p. 168 °C (EtOH). Elemental analysis (%Calc./Found): 63.81/64.53 (C), 5.00/5.51 (H), 19.84/19.84 (N), 11.35/11.35 (S). IR (ν, cm^{-1}): 3500–3350 (NH triazoline); 3043, 3000 (=CH aromatic); 2825, 2828 (CH methylene, asimetric and simetric stretching); 1604, 1567, 1543, 1489, 1350 (C=C and C=N of rings); 1456 (CH methylene bending); 1295 (C–N triazoline); 1261 (C=S); 826 (4-substituted pyridine). ^{1}H NMR, (δ, ppm): 3.01 (2H, t, CH_2–phenyl), 4.36 (2H, t, NCH_2), 6.97–6.99 (2H, m, C_2H and C_6H of phenyl), 7.18–7.22 (3H, m, C_3H, C_4H and C_5H of phenyl), 7.44 (2H, dd, $J = 5.99$ Hz, C_3H and C_5H of pyridine), 8.73 (2H, dd, $J = 5.98$ Hz, C_2H and C_6H of pyridine), 14.15 (1H, s, NH).

*3.1.2. General procedure for the preparation of 5-(4-pyridinyl)-4-substituted-3-(benzoylmethyl)thio-4H-1,2,4-triazoles (**3a–g**)*

Equimolar amounts of **2a–g**, phenacyl chloride and dry potassium carbonate were refluxed in acetone (40 ml) for 8 h. The hot mixture was filtered. The precipitate obtained by cooling was recrystallized from suitable solvent.

*3.1.2.1. 4-Ethyl-5-(4-pyridinyl)-3-(benzoylmethyl)thio-4H-1,2,4-triazole (**3a**).* $C_{17}H_{16}N_4OS$, m.wt. 324.4, pale orange crystals, 68% yield, m.p. 129–30 °C (acetone). Elemental analysis (%Calc./Found): 62.94/63.80 (C), 4.97/5.63 (H), 17.27/17.25 (N), 9.88/9.88% (S). IR (ν, cm^{-1}): 3043, 2989 (=CH aromatic); 2935, 2913 (CH methylene, asimetric and simetric stretching); 1685 (C=O); 1598, 1565, 1478, 1390 (C=C and C=N of rings); 1435 (CH methylene bending); 826 (4-substituted pyridine); 750, 695 (monosubstituted benzene); 641 (C–S). ^{1}H NMR (δ, ppm): 1.28 (3H, t, CH_3), 4.13 (2H, q, NCH_2), 5.03 (2H, s, SCH_2CO), 7.58 (2H, t, $J = 7.69$ Hz, C_3H and C_5H of benzoyl), 7.69–7.72 (3H, m, C_4H of benzoyl and C_3H, C_5H of pyridine), 8.05 (2H, d, C_2H and C_6H of benzoyl), 8.78 (2H, dd, $J = 6.12$ Hz, C_2H and C_6H of pyridine).

*3.1.2.2. 4-Allyl-5-(4-pyridinyl)-3-(benzoylmethyl)thio-4H-1,2,4-triazole (**3b**).* $C_{18}H_{16}N_4OS{\cdot}CH_3OH$, m.wt. 368.5, bright white powder, 70% yield, m.p. 75–77 °C (methanol–water, 1:1). Elemental analysis (%Calc./Found): 61.94/62.12 (C), 5.47/5.67 (H), 15.21/15.91 (N), 8.70/8.46% (S). IR (ν, cm^{-1}): 3475, 3370 (OH), 3043 (=CH aromatic and allyl); 2967, 2913 (CH methylene, asimetric and simetric stretching); 1685 (C=O); 1630, 1608, 1533, 1390 (C=C and C=N of rings, C=C of allyl);

1435 (CH methylene bending); 826 (4-substituted pyridine); 760, 695 (monosubstituted benzene); 640 (C–S). ^{1}H NMR (δ, ppm): 4.78 (2H, NCH_2), 4.89 (1H, d, $J = 17.25$ Hz, C_3H_a of CH=CH_2, *trans*), 5.01 (2H, s, SCH_2CO), 5.27 (1H, d, $J = 10.51$ Hz, C_3H_b of CH=CH_2 *cis*), 5.90–6.14 (1H, m, CH), 7.59 (2H, t, $J = 7.69$ Hz, C_3H and C_5H of benzoyl), 7.66 (2H, dd, $J = 6.08$ Hz, C_3H, C_5H of pyridine), 7.71 (1H, t, $J = 7.40$ Hz, C_4H of benzoyl), 8.04 (2H, d, $J = 8.51$ Hz, C_2H and C_6H of benzoyl), 8.76 (2H, dd, $J = 6.07$ Hz, C_2H and C_6H of pyridine). MS m/z (%): 336 [M^+] (0.17), 105 [phenyl–C≡O$^+$] (100), 104 [pyridine–C≡N]$^{+\cdot}$ (13.5), 91 [phenyl–CH_2]$^+$ (17), 77 [phenyl]$^+$ (93).

*3.1.2.3. 4-Phenyl-5-(4-pyridinyl)-3-(benzoylmethyl)thio-4H-1,2,4-triazole (**3c**).* $C_{21}H_{16}N_4OS$, m.wt. 372.4, creamy white powder, 31% yield, m.p. 175 °C (C_3H_6O). Elemental analysis (%Calc./Found): 67.72/67.32 (C), 4.33/3.72 (H), 15.04/14.69 (N). IR (ν, cm^{-1}): 3054 (=CH aromatic); 2978, 2913 (CH methylene, asimetric and simetric stretching); 1674 (C=O); 1599, 1489,1369 (C=C and C=N of rings); 1435 (CH methylene bending); 826 (4-substituted pyridine); 761, 695 (monosubstituted benzene); 630 (C–S). ^{1}H NMR (δ, ppm): 4.99 (2H, s, CH_2), 7.30 (2H, dd, $J = 6.05$ Hz, C_3H and C_5H of pyridine), 7.49–7.51 (2H, m, C_2H and C_6H of phenyl), 7.56–7.63 (5H, m, C_3H, C_4H and C_5H of phenyl, C_3H and C_5H of benzoyl), 7.70 (1H, t, $J = 7.36$ Hz, C_4H of benzoyl), 8.04 (2H, d, $J = 7.38$ Hz, C_2H and C_6H of benzoyl), 8.57 (2H, dd, $J = 6.04$ Hz, C_2H and C_6H of pyridine).

*3.1.2.4. 4-(4-Bromophenyl)-5-(4-pyridinyl)-3-(benzoylmethyl)thio-4H-1,2,4-triazole (**3d**).* $C_{21}H_{15}BrN_4OS$, m.wt. 451.3, bright red crystals, 76% yield, m.p. 202 °C (EtOH). Elemental analysis (%Calc./Found): 55.88/55.92 (C), 3.35/2.96 (H), 12.41/12.43 (N), 7.10/6.79 (S). IR (ν, cm^{-1}): 3087, 3043 (=CH aromatic); 2974, 2913 (CH methylene, asimetric and simetric stretching); 1700 (C=O); 1610, 1500, 1390 (C=C and C=N of rings); 1435 (CH methylene bending); 1065 (Ar–Br); 826 (1,4-disubstituted benzene and 4-substituted pyridine); 760, 695 (monosubstituted benzene); 640 (C–S). ^{1}H NMR (δ, ppm): 4.97 (2H, s, CH_2), 7.32 (2H, dd, $J = 6.14$ Hz, C_3H and C_5H of pyridine), 7.49 (2H, dd, $J = 8.62$ Hz, C_2H and C_6H of phenyl), 7.58 (2H, t, $J = 7.71$ Hz, C_3H and C_5H of benzoyl), 7.70 (1H, t, $J = 7.39$ Hz, C_4H of benzoyl), 7.82 (2H, dd, $J = 8.62$ Hz, C_3H and C_5H of phenyl), 8.03 (2H, d, $J = 8.50$ Hz, C_2H and C_6H of benzoyl), 8.60 (2H, dd, $J = 6.13$ Hz, C_2H and C_6H of pyridine).

*3.1.2.5. 4-(4-Chlorophenyl)-5-(4-pyridinyl)-3-(benzoylmethyl)thio-4H-1,2,4-triazole (**3e**).* $C_{21}H_{15}ClN_4OS$, m.wt. 406.9, orange crystals, 42% yield, m.p. 181 °C (ethanol). Elemental analysis (%Calc./Found): 61.99/62.98 (C), 3.72/4.13 (H), 13.77/14.01 (N), 7.78/7.29% (S). IR (ν, cm^{-1}): 3054 (=CH aromatic); 2967, 2913 (CH methylene, asimetric and simetric stretching); 1704 (C=O); 1591, 1489, 1369 (C=C and C=N of rings); 1438 (CH methylene bending); 1091 (Ar–Cl); 837 (1,4-disubstituted benzene and 4-substituted pyridine); 750, 695 (monosubstituted benzene); 630 (C–S). ^{1}H NMR (δ, ppm): 4.98 (2H, s, CH_2), 7.32 (2H, dd, $J = 6.15$ Hz, C_3H and C_5H of pyridine), 7.55–7.59 (4H, m, C_3H and C_5H of benzoyl and phenyl), 7.67–7.72 (3H, m, C_4H of benzoyl and C_2H and C_6H of phenyl), 8.03 (2H, d, $J = 8.50$ Hz, C_2H and C_6H of benzoyl), 8.60 (2H, dd, $J = 6.13$ Hz, C_2H and C_6H of pyridine).

*3.1.2.6. 4-(4-Fluorophenyl)-5-(4-pyridinyl)-3-(benzoylmethyl)thio-4H-1,2,4-triazole (**3f**).* $C_{21}H_{15}FN_4OS$, m.wt. 390.4, bright yellow crystals, 70% yield, m.p. 160–161 °C (MeOH). Elemental analysis (%Calc./Found): 64.60/65.39 (C), 3.87/4.09 (H), 14.35/14.41 (N), 8.21/8.25 (S). IR (ν, cm^{-1}): 3065 (=CH aromatic); 2967, 2913 (CH methylene, asimetric and simetric stretching); 1700 (C=O); 1608, 1525, 1390 (C=C and C=N of rings); 1445 (CH methylene bending); 1175 (Ar–F); 848 (1,4-disubstituted benzene and 4-substituted pyridine); 750, 695 (monosubstituted benzene); 640 (C–S). ^{1}H NMR (δ, ppm): 4.81 (2H, s, CH_2), 7.15 (2H, dd, $J = 6.13$ Hz, C_3H and C_5H of pyridine), 7.30 (2H, t, $J = 8.70$ Hz, C_3H and C_5H of phenyl), 7.38–7.44 (4H, m, C_3H and C_5H of benzoyl and C_2H and C_6H of phenyl), 7.54 (1H, t, $J = 7.37$ Hz, C_4H of benzoyl), 7.87 (2H, d, $J = 8.41$ Hz, C_2H and C_6H of benzoyl), 8.43 (2H, dd, $J = 6.13$ Hz, C_2H and C_6H of pyridine). MS m/z (%): 390 [M^+] (0.90), 105 [phenyl–C≡O]$^+$ (95.1), 104 [pyridine–C≡N]$^{+\cdot}$ (4.6), 95 [F–C_6H_5]$^+$ (18.6), 91 [$C_6H_5CH_2$]$^+$ (14), 77 [phenyl]$^+$ (100), 75 [95-HF] (19.9).

*3.1.2.7. 4-Benzyl-5-(4-pyridinyl)-3-(benzoylmethyl)thio-4H-1,2,4-triazole (**3g**).* $C_{22}H_{18}N_4OS$, m.wt. 386.5, white crystals, 46% yield, m.p. 139 °C (C_3H_6O). Elemental analysis (%Calc./Found): 68.37/69.51 (C), 4.69/4.44 (H), 14.50/14.70 (N). IR (ν, cm^{-1}): 3054 (=CH aromatic); 2967, 2923 (CH methylene, asymmetric and symmetric stretching); 1685 (C=O); 1587, 1454, 1348 (C=C and C=N of rings); 1425 (CH methylene bending); 826 (4-substituted pyridine); 735, 674 (monosubstituted benzene); 641 (C–S). ^{1}H NMR (δ, ppm): 4.85 (2H, s, SCH_2CO), 5.26 (2H, s, NCH_2), 6.88 (2H, d, $J = 6.92$ Hz, C_2H and C_6H of benzyl), 7.10–7.19 (3H, m, C_3H, C_4H and C_5H of benzyl), 7.39–7.43 (4H, m, C_3H and C_5H of benzoyl and pyridine), 7.54 (1H, t, $J = 7.39$ Hz, C_4H of benzoyl), 7.87 (2H, d, $J = 7.16$ Hz, C_2H and C_6H of benzoyl), 8.52 (2H, dd, $J = 6.11$ Hz, C_2H and C_6H of pyridine). MS m/z (%): 386 [M^+] (0.77), 105 [phenyl–C≡O]$^+$ (90.6), 104 [pyridine–C≡N]$^{+\cdot}$ (11.5), 91 [$C_6H_5CH_2$]$^+$ (100), 77 [phenyl]$^+$ (91), 65 [C_5H_5]$^+$ (38.1).

3.2. Microbiology

3.2.1. In vitro evaluation of antibacterial and antifungal activities

Tested compounds and control antibiotics were dissolved in DMSO and sterile distilled water, respectively for the preparation of stock solutions. Further dilutions were made in sterile distilled water. The in vitro antimicrobial activities of the tested compounds were carried out by the paper disc diffusion method [17]. Overnight cultures of microorganisms were adjusted to ca. 10^6 c.f.u. ml^{-1} according to the Mac Farland turbidity standards [18] and spread over the appropriate media (Mueller–Hinton agar for bacteria, same medium supplemented with 5% blood for *Enterococcus faecalis*, Sabouraud Dextrose Agar for the yeast), in petri dishes. Filter paper discs (ϕ 5 mm) were placed on the surface of the media and the solutions containing 500 μg of the compounds were dropped on the discs. Discs containing DMSO were also used as control. After overnight incubation at 37 °C, the zones of inhibition around the discs were measured. The antimicrobial effects of the compounds that produced ≥ 12 mm zones of inhibition were tested quantitatively in respective broth media by using macrodilution method and the MIC (minimal inhibitory concentration) values (μg ml^{-1}) were determined [19]. The same test was carried out with DMSO as control, as well as with the control antibiotics. For *candida albicans* RPMI 1640 medium buffered with MOPS was used for MIC determination [20].

3.2.2. In vitro evaluation of antimycobacterial activity against Mycobacterium tuberculosis $H_{37}Rv$

A primary screen was conducted at 6.25 μg ml^{-1} (or molar equivalent of highest molecular weight compound in a series of congeners) against *M. tuberculosis* $H_{37}Rv$ in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA). Since their inhibitions were less than 90% in the primary screen, compounds were not evaluated further [21,22].

4. Results and discussion

Table 1 shows the antimicrobial activity of some of the above mentioned compounds against a variety of bacteria. Known antibacterial and antifungal antibiotics (cefotaxime and fluconazole, respectively) were included to compare the results. DMSO which used in dissolving of the compounds, had no effect on microorganisms. The antimicrobial activity of the compounds was tested on the Gram positive strains *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC 12228, *E. faecalis* ATCC 29212; the Gram negative strains *Proteus mirabilis* ATCC 14153, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 4352, *Pseudomonas aeruginosa* ATCC 27853, and the yeast strain *C. albicans* ATCC 10231.

As summarized in Table 1, compounds did not exhibit activity against gram-positive and -negative bacteria. The commonly used β-lactam antibiotic cefotaxime was highly effective against *B. subtilis*, *S. aureus*, *S. epidermidis*, *E. faecalis*, *P. mirabilis*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* and its MIC values were 0.195, 1.56, 0.39, 156.2, 0.024, 0.097, 0.195 and 78.1 μg ml^{-1}, respectively. The reference antifungal flucanozale was highly active against *C. albicans* and its MIC value was 0.5 μg ml^{-1}, while those of compounds **1c**, **2a**, **3a**, **3c** and **3g** were 50, 50, 25, 25 and 50 μg ml^{-1}, respectively.

The MIC values for resistance are ≥ 64, ≥ 1 and ≥ 32 μg ml^{-1} for commonly used antifungals fluconazole, itraconazole and flucytosine, respectively. The value for intermediate activity for flucytosine is 8–16 μg ml^{-1} [20]. The antifungal activity of our compounds **3a** and **3c** fall between intermediate and resistant category when compared with flucytosine. *C. albicans* ATCC 10231 was not as resistant to compounds **1c**, **2a**, **3a**, **3c** and **3g** as to fluconazole.

According to primary antituberculosis activity results, the MIC values of compounds **2f**, **2h** and **3a**–**g** were higher than 6.25 μg ml^{-1}, while the MIC value of reference rifampicin was 0.25 μg ml^{-1}. Compounds **2f**, **3b**–**d** showed low inhibition (2, 6, 12 and 16%, respectively).

Acknowledgements

We thank the Research Fund of Marmara University which supported this work (project number SB-40/96) and Dr Joseph A. Maddry from the Tuberculosis Antimicrobial Acquisition and Coordinating Facility

Table 1
Antibacterial activity of some of the compounds (MIC, μg ml^{-1})

MIC	*B. subtilis*	*S. aureus*	*S. epidermidis*	*E. faecalis*	*P. mirabilis*	*E. coli*	*K. pneumoniae*	*P. aeruginosa*
250	**1a**, **1c**, **1d**, **1f**, **2a–d**, **3b**	**2f**	**1d**, **1e**, **2d**, **2f**	**1d**, **2a**, **2c**, **2d**, **2g**, **2h**, **3f**		**1c**, **1d**, **1e**, **2a**, **3b**	**1c**, **1d**	**1c**, **1d**
500	**1e**, **2f**, **3a**			**3b**	**1d**, **1e**, **2a**		**2a**, **2d**	

(TAACF), National Institute of Allergy and Infectious Diseases Southern Research Institute, GWL Hansen's Disease Center, Colorado State University, Birmingham, Alabama, USA, for the in vitro evaluation of antimycobacterial activity.

References

[1] B.N. Goswami, J.C.S. Kataky, J.N. Baruah, Synthesis and antibacterial activity of 1-(2,4-dichlorobenzoyl)-4-substituted thiosemicarbazides, 1,2,4-triazoles and their methyl derivatives, J. Heterocycl. Chem. 21 (1984) 1225–1229.

[2] A.I. Eid, F.A. Ragab, S.L. El-Ansary, S.M. El-Gazayerly, F.E. Mourad, Synthesis of new 7-substituted-4-methylcoumarin derivatives of antimicrobial activity, Arch. Pharm. (Weinheim) 327 (1994) 211–213.

[3] N. Kalyoncuoğlu, S. Rollas, D. Sür-Altıner, Y. Yeğenoğlu, Ö. Anğ, 1[*p*-(Benzoylamino)benzoyl]-4-substituted thiosemicarbazides: synthesis and antibacterial and antifungal activities, Pharmazie 47 (1992) 796–797.

[4] S. Rollas, N. Kalyoncuoğlu, D. Sür-Altıner, Y. Yeğenoğlu, 5-(4-Aminophenyl)-4-substituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones: synthesis and antibacterial and antifungal activities, Pharmazie 48 (1993) 308–309.

[5] I. Mir, M.T. Siddiqui, A. Comrie, Antituberculosis agents-I. α-[5-(2-Furyl)-1,2,4-triazole-3-ylthio]acethydrazide and related compounds, Tetrahedron 26 (1970) 5235–5238.

[6] S. Bahadur, A.K. Goel, Potential antimycobacterial agents, Part II: Synthesis of *N*-1-(2-anilinobenzoyl)-*N*-4-aryl-thiosemicarbazides and semicarbazides, Indian J. Pharm. 38 (1976) 71–73.

[7] I.I. Ismail, A. Mandour, F. Abd El-Azeem, Some semicarbazide derivatives and their antimicrobial activity, Orient. J. Chem. 8 (1992) 160–164 ref.: C.A. 117 (1992) 111215c.

[8] Goodman, Gilman, in: J.G. Hardman, A.G. Gilman, L.E. Limbird (Eds.), The Pharmacological Basis of Therapeutics, 9th ed., McGraw-Hill, New York, 1996, pp. 1155–1156, 1166.

[9] A.A.B. Hazzaa, F. Ashour, R.M. Shafik, Synthesis of 1,2,4-triazolylthiopropanol-amines as potential cardiovascular agents, Pharmazie 35 (1980) 324–325.

[10] I.Y. Postovskii, N.N. Vereshchagina, Z. Obsc. Chim. 26 (1956) 2583 ref.: C.A. 51 (1957) 5055b.

[11] C. Demetrescu, V. Manu, C. Chirita, Neue N^1-acyl-N^4-arylthiosemicarbazide von therapeutischem interesse, Pharmazie 25 (1970) 317–319.

[12] S. Rollas, Synthesis and spectrometric analysis of some 1,2,4-triazoline-5-thiones, J. Fac. Pharm. İstanbul 17 (1981) 155–163.

[13] E. Hassan, M.I. Al-Ashmawi, B. Abdel-Fattah, Synthesis and antimicrobial testing of certain oxadiazoline and triazole derivatives, Pharmazie 38 (1983) 833–835.

[14] R.M. Silverstein, G.C. Bassler, T.C. Morrill, Spectrometric Identification of Organic Compounds, 5th ed., John Wiley and Sons Inc., Singapore, 1991, pp. 103–128.

[15] N. Gülerman, S. Rollas, M. Kiraz, A.C. Ekinci, A. Vidin, Evaluation of antimycobacterial and anticonvulsant activities of new 1-(4-fluorobenzoyl)-4-substituted-thiosemicarbazide and 5-(4-fluorophenyl)-4-substituted-2,4,dihydro-3*H*-1,2,4-triazole-3-thione derivatives, Farmaco 52 (1997) 691–695.

[16] L.T. Creagh, P. Truitt, Nuclear magnetic resonance studies of triazoles. I. Tautomerism of 1,2,4-triazole, J. Org. Chem. 33 (1968) 2956–2957.

[17] Performance Standards for Antimicrobial Disc Susceptibility Tests, 6th ed., Approved Standard, NCCLS, Document M2-A6, 17(1), National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania, 1997.

[18] V. Lorian (Ed.), Antibiotics in Laboratory Medicine, 2nd ed., Williams & Wilkins, London, 1986, p. 116.

[19] Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, 4th ed., Approved Standard, NCCLS Document M7-A4, 17 (2), Wayne, Pennsylvania, 1997.

[20] Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard, NCCLS Document M 27-A, 17(9), Wayne, Pennsylvania, 1997.

[21] C.B. Inderleid, in: V. Lorian (Ed.), Antibiotics in Laboratory Medicine, 3rd ed., Williams and Wilkins, Baltimore, 1991, p. 134.

[22] Ş.G. Küçükgüzel, S. Rollas, İ. Küçükgüzel, M. Kiraz, Synthesis and antimycobacterial activity of some coupling products from 4-aminobenzoic acid hydrazones, Eur. J. Med. Chem. 34 (1999) 1093–1100.