Hindawi Publishing Corporation
Journal of Chemistry
Volume 2016, Article ID 1692540, 7 pages
http://dx.doi.org/10.1155/2016/1692540

Research Article

Synthesis and *In Vitro* Evaluation of New Thiosemicarbazone Derivatives as Potential Antimicrobial Agents

Zafer Asım Kaplancıklı,[1] Mehlika Dilek Altıntop,[1] Belgin Sever,[1] Zerrin Cantürk,[2] and Ahmet Özdemir[1]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey*
[2]*Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey*

Correspondence should be addressed to Zafer Asım Kaplancıklı; zakaplan@anadolu.edu.tr

Received 16 December 2015; Revised 10 January 2016; Accepted 13 January 2016

Academic Editor: Giuseppe Gumina

In an effort to develop potent antimicrobial agents, new thiosemicarbazone derivatives were synthesized *via* the reaction of 4-[4-(trifluoromethyl)phenyl]thiosemicarbazide with aromatic aldehydes. The compounds were evaluated for their inhibitory effects on pathogenic bacteria and yeasts using the CLSI broth microdilution method. Microplate Alamar Blue Assay was also carried out to determine the antimycobacterial activities of the compounds against *Mycobacterium tuberculosis* H37Rv. Among these derivatives, compounds **5** and **11** were more effective against *Enterococcus faecalis* (ATCC 29212) than chloramphenicol, whereas compounds **1**, **2**, and **12** and chloramphenicol showed the same level of antibacterial activity against *E. faecalis*. Moreover, compound **2** and chloramphenicol exhibited the same level of antibacterial activity against *Staphylococcus aureus*. On the other hand, the most potent anticandidal derivatives were found as compounds **2** and **5**. These derivatives and ketoconazole exhibited the same level of antifungal activity against *Candida glabrata*. According to the Microplate Alamar Blue Assay, the tested compounds showed weak to moderate antitubercular activity.

1. Introduction

Throughout history, it has been a major worldwide problem to treat microbial diseases caused by bacteria and fungi due to impetuous development of resistance to antibacterial and antifungal drugs. Antimicrobial resistance is observed in pathogenic bacteria before exposure to antimicrobial agent or related to uncontrolled infection after a patient receives that antimicrobial. Bacteria could be resistant inherently staying in variable temperatures and in highly acidic or alkaline conditions or could develop resistance by *de novo* mutation or through the transmission of resistance genes from other organisms. Gram-positive bacterial infections particularly caused by *Staphylococcus aureus* and *Enterococcus* spp. represent a major health problem hampering the therapeutic efficacy of antibacterial drugs due to their frequent multidrug resistance [1–6].

In the past two decades, the incidence of fungal infections has gone up all over the world. Fungal infections classified as allergic reactions to fungal proteins and toxic reactions to fungi toxins and mycoses pose a constant and serious threat to human life. Infections caused by *Candida* species represent the main reason of opportunistic fungal infections worldwide. Candidemia and other forms of invasive candidiasis are important causes of the increasing rate of morbidity and mortality. *Candida albicans* is the most common etiological agent of candidiasis and *Candida glabrata* is a growing concern in clinical settings because it causes mucosal and systemic bloodstream infections [7–12].

The World Health Organization (WHO) evaluates tuberculosis as one of the deadliest global diseases associated with the occurrence of multiple drug resistant strains of *Mycobacterium tuberculosis* discovered by Robert Koch in 1882. *Mycobacterium tuberculosis* H37Rv, the pathogenic agent of tuberculosis, is recognized to reproduce in host cells by pulling through host cell defenses supplied by macrophages and dendritic cells. In 2013, approximately 9 million people were diagnosed with active tuberculosis

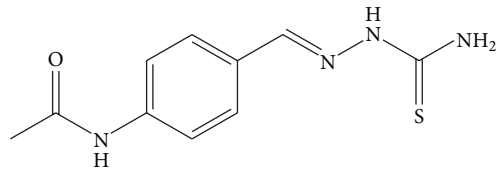


Figure 1: Thiacetazone.

and 1.5 million deaths attributed to this disease. Diagnosis and treatment are crucial for tuberculosis, particularly in high-risk populations. Early diagnosis of tuberculosis with radiography and computed tomography methods is essential for effective treatment and leads to a reduced onward transmission of tuberculosis. Despite the discovery of tuberculin test and Bacillus-Calmette Guérin (BCG) vaccine and some main drugs called streptomycin, isoniazid, and rifampicin for the treatment of tuberculosis, it has remained one of the most dangerous diseases especially associated with mycobacterial resistance in chemotherapy and the presence of coinfectious diseases such as acquired immune deficiency syndrome (AIDS) [8–18].

Thiosemicarbazones have been investigated for medicinal studies for a long while due to their wide range of biological activities including antineoplastic, antimycobacterial, antibacterial, antifungal, antiviral, and antimalarial effects and versatility as nitrogen and sulfur donors allowing them to bring on a great variety of coordination modes. Thiosemicarbazone is also known as an iron-chelating group, bonding the sulfur and azomethine nitrogen atoms. The complexes of nitrogen and sulfur atoms with metal ions may be considered potential biological agents. In recent years, many thiosemicarbazone derivatives have been synthesized and evaluated for their antibacterial activity. The current use of these agents in bacterial infections has led to the development of novel antibacterial drugs [19–25].

Thiacetazone, *p*-acetamidobenzaldehyde thiosemicarbazone, is one of the oldest and cheapest agents used as a second line drug for tuberculosis treatment (Figure 1). This agent is estimated to show antimycobacterial activity inhibiting mycolic acid biosynthesis. Thiacetazone shows only bacteriostatic activity and develops resistance easily during tuberculosis therapy. It is partly cross-resistant to ethionamide and it is forbidden to be used in patients with human immunodeficiency virus (HIV) owing to high frequency of Stevens-Johnson syndrome [26–28].

On the basis of aforementioned findings, herein, we synthesized a new series of thiosemicarbazone derivatives and investigated their *in vitro* antimicrobial and antimycobacterial effects.

2. Experimental Section

2.1. Chemistry. All reagents were purchased from commercial suppliers and were used without further purification. Melting points (Mp) were determined on an Electrothermal 9100 melting point apparatus (Weiss-Gallenkamp, Loughborough, UK) and are uncorrected. IR spectra were recorded on an IRPrestige-21 Fourier Transform Infrared spectrophotometer (Shimadzu, Tokyo, Japan). ^{1}H NMR spectra were recorded on a Bruker spectrometer (Bruker, Billerica, MA, USA). Mass spectra were recorded on an Agilent LC-MSD-Trap-SL Mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Elemental analyses (C, H, and N) were performed on a Perkin Elmer EAL 240 elemental analyzer (Perkin-Elmer, Norwalk, CT, USA) and the results were within ±0.4% of the theoretical values. Thin Layer Chromatography (TLC) was performed on TLC Silica gel 60 F_{254} aluminium sheets (Merck, Darmstadt, Germany) to check the purity of the compounds.

2.2. General Procedure for the Synthesis of the Compounds

2.2.1. 4-[4-(Trifluoromethyl)phenyl]thiosemicarbazide. A mixture of 4-(trifluoromethyl)phenyl isothiocyanate (0.1 mol) and hydrazine hydrate (0.2 mol) in ethanol (30 mL) was stirred at room temperature for 4 h and then filtered. The residue was crystallized from ethanol [29].

*2.2.2. 4-[4-(Trifluoromethyl)phenyl]-1-(substituted benzylidene)thiosemicarbazides (**1**–**12**).* A mixture of 4-[4-(trifluoromethyl)phenyl]thiosemicarbazide (0.01 mol) and aromatic aldehyde (0.01 mol) was refluxed in ethanol for 6 h, filtered, and crystallized from ethanol [29].

*2.2.3. 4-[4-(Trifluoromethyl)phenyl]-1-(benzylidene)thiosemicarbazide (**1**).* IR ν_{max} (cm^{-1}): 3313.71 (N-H stretching), 3140.11 (aromatic C-H stretching), 1614.42, 1539.20, 1504.48 (C=N, C=C stretching and N-H bending), 1327.03, 1278.81, 1099.43 (C-N, C=S stretching and aromatic C-H in plane bending), 846.75, 754.17, 686.66 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.43–7.45 (m, 3H, aromatic protons), 7.72 (d, J = 10 Hz, 2H, aromatic protons), 7.89–7.91 (m, 4H, aromatic protons), 8.19 (s, 1H, CH=N), 10.29 (s, 1H, N-H), 12.03 (s, 1H, N-H); MS (ESI) (m/z): (M + H)$^+$ 324; Anal. Calcd for $C_{15}H_{12}F_3N_3S$: C, 55.72; H, 3.74; N, 13.00; Found: C, 55.70; H, 3.76; N, 13.00.

*2.2.4. 4-[4-(Trifluoromethyl)phenyl]-1-(4-fluorobenzylidene)thiosemicarbazide (**2**).* IR ν_{max} (cm^{-1}): 3288.63 (N-H stretching), 3153.61 (aromatic C-H stretching), 2983.88 (C-H stretching), 1616.35, 1600.92, 1544.98, 1504.48 (C=N, C=C stretching and N-H bending), 1319.31, 1269.16, 1232.51, 1192.01, 1161.15, 1109.07, 1064.71, 1014.56 (C-N, C=S stretching and aromatic C-H in plane bending), 831.32 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.62–7.63 (m, 2H, aromatic protons), 7.72 (d, J = 10 Hz, 2H, aromatic protons), 7.89 (d, J = 10 Hz, 2H, aromatic protons), 7.97–8.01 (m, 2H, aromatic protons), 8.18 (s, 1H, CH=N), 10.31 (s, 1H, N-H), 12.03 (s, 1H, N-H); MS (ESI) (m/z): (M + H)$^+$ 342; Anal. Calcd for $C_{15}H_{11}F_4N_3S$: C, 52.78; H, 3.25; N, 12.31; Found: C, 52.80; H, 3.24; N, 12.30.

*2.2.5. 4-[4-(Trifluoromethyl)phenyl]-1-(4-chlorobenzylidene)thiosemicarbazide (**3**).* IR ν_{max} (cm^{-1}): 3329.14 (N-H stretching), 3134.33 (aromatic C-H stretching), 2980.02 (C-H stretching), 1616.35, 1593.20, 1541.12, 1489.05 (C=N, C=C

stretching and N-H bending), 1321.24, 1274.95, 1109.07, 1085.92, 1062.78, 1012.63 (C-N, C=S stretching and aromatic C-H in plane bending), 823.60 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.49–7.51 (m, 2H, aromatic protons), 7.73 (d, J = 10 Hz, 2H, aromatic protons), 7.88 (d, J = 10 Hz, 2H, aromatic protons), 7.94–7.96 (m, 2H, aromatic protons), 8.16 (s, 1H, CH=N), 10.34 (s, 1H, N-H), 12.07 (s, 1H, N-H); MS (ESI) (m/z): $(M + H)^+$ 358; Anal. Calcd for $C_{15}H_{11}ClF_3N_3S$: C, 50.36; H, 3.10; N, 11.74; Found: C, 50.35; H, 3.10; N, 11.75.

*2.2.6. 4-[4-(Trifluoromethyl)phenyl]-1-(4-bromobenzylidene)thiosemicarbazide (**4**).* IR ν_{max} (cm^{-1}): 3329.14 (N-H stretching), 3142.04 (aromatic C-H stretching), 2981.95 (C-H stretching), 1614.42, 1589.34, 1539.20, 1521.84, 1487.12 (C=N, C=C stretching and N-H bending), 1323.17, 1273.02, 1207.44, 1165.00, 1107.14, 1062.78, 1006.84 (C-N, C=S stretching and aromatic C-H in plane bending), 840.96, 819.75, 808.17 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.62–7.64 (m, 2H, aromatic protons), 7.73 (d, J = 5 Hz, 2H, aromatic protons), 7.88 (d, J = 5 Hz, 4H, aromatic protons), 8.15 (s, 1H, CH=N), 10.34 (s, 1H, N-H), 12.07 (s, 1H, N-H); MS (ESI) (m/z): $(M + H)^+$ 403; Anal. Calcd for $C_{15}H_{11}BrF_3N_3S$: C, 44.79; H, 2.76; N, 10.45; Found: C, 44.78; H, 2.74; N, 10.46.

*2.2.7. 4-[4-(Trifluoromethyl)phenyl]-1-(4-nitrobenzylidene)thiosemicarbazide (**5**).* IR ν_{max} (cm^{-1}): 3307.92 (N-H stretching), 3116.97 (aromatic C-H stretching), 2980.02 (C-H stretching), 1614.42, 1541.12, 1516.05 (C=N, C=C stretching and N-H bending), 1330.88, 1317.38, 1188.15, 1105.21, 1066.64 (C-N, C=S stretching and aromatic C-H in plane bending), 837.11 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.75 (d, J = 5 Hz, 2H, aromatic protons), 7.87 (d, J = 10 Hz, 2H, aromatic protons), 8.18–8.20 (m, 2H, aromatic protons), 8.24–8.27 (m, 3H, CH=N, aromatic protons), 10.48 (s, 1H, N-H), 12.28 (s, 1H, N-H); MS (ESI) (m/z): $(M + H)^+$ 369; Anal. Calcd for $C_{15}H_{11}F_3N_4O_2S$: C, 48.91; H, 3.01; N, 15.21; Found: C, 48.90; H, 3.03; N, 15.20.

*2.2.8. 4-[4-(Trifluoromethyl)phenyl]-1-(4-hydroxybenzylidene)thiosemicarbazide (**6**).* IR ν_{max} (cm^{-1}): 3367.71, 3286.70 (N-H stretching), 3165.19 (aromatic C-H stretching), 1604.77, 1541.12, 1510.26, 1494.83 (C=N, C=C stretching and N-H bending), 1327.03, 1276.88, 1186.22, 1163.08, 1099.43, 1064.71, 1018.41 (C-N, C-O, C=S stretching and aromatic C-H in plane bending), 831.32 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 6.80–6.83 (m, 2H, aromatic protons), 7.70–7.74 (m, 4H, aromatic protons), 7.92 (d, J = 10 Hz, 2H, aromatic protons), 8.09 (s, 1H, CH=N), 9.97 (s, 1H, O-H), 10.17 (s, 1H, N-H), 11.85 (s, 1H, N-H); MS (ESI) (m/z): $(M + H)^+$ 340; Anal. Calcd for $C_{15}H_{12}F_3N_3OS$: C, 53.09; H, 3.56; N, 12.38; Found: C, 53.10; H, 3.55; N, 12.37.

*2.2.9. 4-[4-(Trifluoromethyl)phenyl]-1-(4-methoxybenzylidene)thiosemicarbazide (**7**).* IR ν_{max} (cm^{-1}): 3142.04 (aromatic C-H stretching), 2978.09 (C-H stretching), 1606.70, 1541.12, 1490.97 (C=N, C=C stretching and N-H bending), 1246.02, 1207.44, 1165.00, 1107.14, 1060.85, 1028.06, 1016.49 (C-N, C=S stretching and aromatic C-H in plane bending), 829.39, 792.74 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 3.80 (s, 3H, OCH_3), 6.98–7.00 (m, 2H, aromatic protons), 7.71 (d, J = 10 Hz, 2H, aromatic protons), 7.83–7.86 (m, 2H, aromatic protons), 7.91 (d, J = 10 Hz, 2H, aromatic protons), 8.05 and 8.14 (2s, 1H, CH=N), 10.22 and 10.35 (2s, 1H, N-H), 11.77 and 11.92 (2s, 1H, N-H); MS (ESI) (m/z): $(M + H)^+$ 354; Anal. Calcd for $C_{16}H_{14}F_3N_3OS$: C, 54.38; H, 3.99; N, 11.89; Found: C, 54.37; H, 3.98; N, 11.91.

*2.2.10. 4-[4-(Trifluoromethyl)phenyl]-1-(4-methylbenzylidene)thiosemicarbazide (**8**).* IR ν_{max} (cm^{-1}): 3309.85 (N-H stretching), 3140.11 (aromatic C-H stretching), 2978.09 (C-H stretching), 1616.35, 1541.12, 1492.90 (C=N, C=C stretching and N-H bending), 1325.10, 1278.81, 1109.07, 1062.78 (C-N, C=S stretching and aromatic C-H in plane bending), 806.25 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H, CH_3), 7.25 (d, J = 10 Hz, 2H, aromatic protons), 7.72 (d, J = 10 Hz, 2H, aromatic protons), 7.79 (d, J = 10 Hz, 2H, aromatic protons), 7.90 (d, J = 10 Hz, 2H, aromatic protons), 8.06 and 8.15 (2s, 1H, CH=N), 10.26 and 10.38 (2s, 1H, N-H), 11.81 and 11.96 (2s, 1H, N-H); MS (ESI) (m/z): $(M + H)^+$ 338; Anal. Calcd for $C_{16}H_{14}F_3N_3S$: C, 56.96; H, 4.18; N, 12.46; Found: C, 56.95; H, 4.17; N, 12.45.

*2.2.11. 4-[4-(Trifluoromethyl)phenyl]-1-(4-isopropylbenzylidene)thiosemicarbazide (**9**).* IR ν_{max} (cm^{-1}): 3290.56 (N-H stretching), 3140.11 (aromatic C-H stretching), 2976.16 (C-H stretching), 1616.35, 1541.12, 1492.90 (C=N, C=C stretching and N-H bending), 1323.17, 1276.88, 1107.14, 1064.71 (C-N, C=S stretching and aromatic C-H in plane bending), 839.03, 817.32 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.21 (d, J = 5 Hz, 6H, $2CH_3$), 2.89–2.95 (m, 1H, CH, i-Pr), 7.31 (d, J = 5 Hz, 2H, aromatic protons), 7.72 (d, J = 10 Hz, 2H, aromatic protons), 7.81 (d, J = 10 Hz, 2H, aromatic protons), 7.90 (d, J = 10 Hz, 2H, aromatic protons), 8.07 and 8.15 (2s, 1H, CH=N), 10.25 and 10.39 (2s, 1H, N-H), 11.83 and 11.97 (2s, 1H, N-H); MS (ESI) (m/z): $(M + H)^+$ 366; Anal. Calcd for $C_{18}H_{18}F_3N_3S$: C, 59.16; H, 4.97; N, 11.50; Found: C, 59.17; H, 4.96; N, 11.51.

*2.2.12. 4-[4-(Trifluoromethyl)phenyl]-1-(4-dimethylaminobenzylidene)thiosemicarbazide (**10**).* IR ν_{max} (cm^{-1}): 3275.13 (N-H stretching), 3140.11 (aromatic C-H stretching), 2978.09 (C-H stretching), 1598.99, 1548.84, 1519.91, 1492.90 (C=N, C=C stretching and N-H bending), 1325.10, 1315.45, 1276.88, 1161.15, 1105.21, 1060.85 (C-N, C=S stretching and aromatic C-H in plane bending), 943.19, 840.96, 810.10, 786.96 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.97 (s, 6H, $N(CH_3)_2$), 6.72 (d, J = 10 Hz, 2H, aromatic protons), 7.68–7.71 (m, 4H, aromatic protons), 7.94 (d, J = 10 Hz, 2H, aromatic protons), 8.07 (s, 1H, CH=N), 10.12 (s, 1H, N-H), 11.79 (s, 1H, N-H); MS (ESI) (m/z): (M +

H)$^+$ 367; Anal. Calcd for $C_{17}H_{17}F_3N_4S$: C, 55.73; H, 4.68; N, 15.29. Found: C, 55.72; H, 4.69; N, 15.26.

*2.2.13. 4-[4-(Trifluoromethyl)phenyl]-1-(4-trifluoromethylbenzylidene)thiosemicarbazide (**11**).* IR ν_{max} (cm^{-1}): 3132.40 (aromatic C-H stretching), 2985.81, 2883.58 (C-H stretching), 1598.99, 1541.12, 1492.90 (C=N, C=C stretching and N-H bending), 1394.53, 1319.31, 1064.71 (C-N, C=S stretching and aromatic C-H in plane bending), 825.53 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.76 (dd, J = 10 Hz, 20 Hz, 4H, aromatic protons), 7.88 (d, J = 5 Hz, 2H, aromatic protons), 8.14 (d, J = 10 Hz, 2H, aromatic protons), 8.24 (s, 1H, CH=N), 10.42 and 10.62 (2s, 1H, N-H), 12.02 and 12.18 (2s, 1H, N-H); MS (ESI) (m/z): (M + H)$^+$ 392; Anal. Calcd for $C_{16}H_{11}F_6N_3S$: C, 49.11; H, 2.83; N, 10.74; Found: C, 49.10; H, 2.82; N, 10.76.

*2.2.14. 4-[4-(Trifluoromethyl)phenyl]-1-(4-cyanobenzylidene)thiosemicarbazide (**12**).* IR ν_{max} (cm^{-1}): 3309.85 (N-H stretching), 3116.97 (aromatic C-H stretching), 2978.09, 2883.58 (C-H stretching), 2220.07 (C≡N stretching), 1541.12, 1521.84, 1498.69 (C=N, C=C stretching and N-H bending), 1325.10, 1269.16, 1190.08, 1166.93, 1105.21, 1087.85, 1066.64, 1016.49 (C-N, C=S stretching and aromatic C-H in plane bending), 921.97, 831.32 (aromatic C-H out of plane bending); ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.74 (d, J = 5 Hz, 2H, aromatic protons), 7.86–7.90 (m, 4H, aromatic protons), 8.12 (d, J = 10 Hz, 2H, aromatic protons), 8.20 (s, 1H, CH=N), 10.43 (s, 1H, N-H), 12.22 (s, 1H, N-H); MS (ESI) (m/z): (M + H)$^+$ 349; Anal. Calcd for $C_{16}H_{11}F_3N_4S$: C, 55.17; H, 3.18; N, 16.08; Found: C, 55.15; H, 3.19; N, 16.07.

2.3. Microbiology

2.3.1. In Vitro Evaluation of Antimicrobial Activity. The microbiological assay was carried out according to the CLSI reference M7-A7 broth microdilution method as described previously [30]. Chloramphenicol and ketoconazole were used as reference agents.

Compounds **1–12** were investigated for their *in vitro* growth inhibitory activity against pathogenic bacteria and fungi. Microorganisms used in this study were as follows: *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Enterococcus faecalis* (ATCC 51922), *Listeria monocytogenes* (ATCC 1911), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 35218), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), and *Candida parapsilosis* (ATCC 22019).

2.3.2. Microplate Alamar Blue Assay (MABA) for Antitubercular Activity. *Mycobacterium tuberculosis* H37Rv (ATCC 27294) was provided from American Type Culture Collection (ATCC) cell bank. The cells were grown in ATCC® Medium 1395: Middlebrook 7H9 broth with ADC enrichment at 37°C for 30 days. The turbidity of the cultures was adjusted to McFarland standard number 1. The following concentrations of the compounds and rifampicin (Sigma, R3501, China) were used: 1.5625–800 μg/mL. All black, clear-bottomed, 96-well plates (Corning 3340, USA) were incubated at 37°C in 5% CO_2 for 7 days. On day 7 of the incubation, freshly prepared 1 : 1 mixture of Alamar Blue reagent (1 : 10 dilution, Invitrogen, 1025, USA) and 10% Tween 80 was added to one well among the positive controls. The plates were further incubated at 37°C for 24 h. If the contents of the well turned pink, the reagent mixture was added to all the wells of the microplate.

Table 1: The yields and melting points (Mp) of the thiosemicarbazone derivatives (**1–12**).

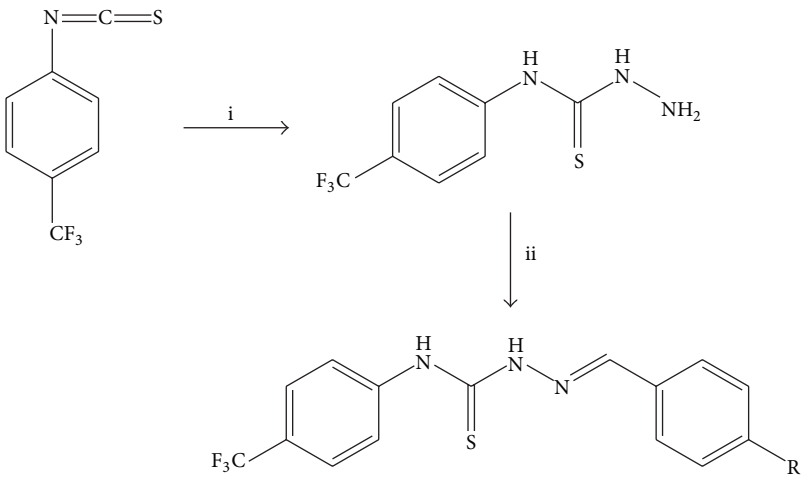
Compound	R	Yield (%)	Mp (°C)
1	H	80	198
2	F	82	169
3	Cl	87	211
4	Br	90	224
5	NO_2	94	233
6	OH	72	204
7	OCH_3	78	200
8	CH_3	79	203
9	$CH(CH_3)_2$	72	180
10	$N(CH_3)_2$	83	214
11	CF_3	80	205
12	CN	96	232

3. Results and Discussion

The synthesis of new thiosemicarbazone derivatives (**1–12**) was performed as described in Scheme 1. 4-[4-(Trifluoromethyl)phenyl]thiosemicarbazide was synthesized *via* the reaction of 4-(trifluoromethyl)phenyl isothiocyanate with hydrazine hydrate. The treatment of 4-[4-(trifluoromethyl)phenyl]thiosemicarbazide with aromatic aldehydes afforded the thiosemicarbazone derivatives (**1–12**). The yields and melting points (Mp) of the compounds were given in Table 1.

The structures of new compounds were confirmed by spectroscopic data and elemental analysis. In the IR spectra of compounds **1–12**, the N-H stretching bands were observed in the region 3367–3275 cm^{-1}. The aromatic C-H stretching vibrations gave rise to bands at 3165–3116 cm^{-1}. C=N, C=C stretching and N-H bending bands were observed in the region 1616–1487 cm^{-1}. In the IR spectra of compound **12**, the stretching band for C≡N group occurred at 2220.07 cm^{-1}.

In the ^{1}H NMR spectra of compounds **1–12**, the signal due to the CH=N proton was observed in the region 8.0–8.3 ppm. The N-H protons appeared in the region 10–13 ppm. In the ^{1}H NMR spectra of some compounds, N-H and CH=N protons gave rise to two singlet peaks in accordance with the presence of the *E* and *Z* isomers [31]. Other aromatic and aliphatic protons were observed at expected regions. The mass spectral data of the synthesized compounds were found in full agreement with the proposed structures.



1–12

Scheme 1: The synthetic route for the preparation of the thiosemicarbazone derivatives (**1**–**12**). Reagents and conditions: (i) $NH_2NH_2{\cdot}H_2O$, ethanol, rt, 4 h; (ii) ArCHO, ethanol, reflux, 6 h.

Table 2: Antibacterial activity of the thiosemicarbazone derivatives (MIC values in μg/mL).

Compound	A	B	C	D	E	F	G	H	I
1	400	200	400	400	400	400	400	400	200
2	100	200	1600	1600	800	1600	1600	400	400
3	400	400	400	800	400	400	400	400	400
4	400	400	400	400	400	400	800	400	400
5	400	100	400	400	400	400	400	400	400
6	400	400	400	400	400	400	400	400	400
7	400	400	400	800	400	400	800	400	200
8	400	400	400	800	400	400	800	400	200
9	400	400	400	400	400	400	800	400	200
10	400	400	400	400	400	400	400	400	200
11	400	100	400	800	400	400	400	400	200
12	400	200	400	400	400	400	400	400	200
Chloramphenicol	100	200	200	100	200	200	100	25	Nt
Rifampicin	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	25

A: *S. aureus* (ATCC 25923), B: *E. faecalis* (ATCC 29212), C: *E. faecalis* (ATCC 51922), D: *L. monocytogenes* (ATCC 1911), E: *K. pneumoniae* (ATCC 700603), F: *P. aeruginosa* (ATCC 27853), G: *E. coli* (ATCC 35218), H: *E. coli* (ATCC 25922), and I: *M. tuberculosis* H37Rv.
Nt: not tested.

The compounds were tested *in vitro* against a number of Gram-positive and Gram-negative bacteria and yeasts using broth microdilution method. Based on this assay, the minimum inhibitory concentrations (MICs) of the compounds were determined. The observed MIC values were in the range of 100–1600 μg/mL. In general, the tested compounds exhibited more potent inhibitory activity towards Gram-positive bacteria compared to Gram-negative bacteria (Table 2).

Among these derivatives, fluorosubstituted compound **2** exhibited the highest antibacterial activity against *S. aureus* with a MIC value of 100 μg/mL when compared with chloramphenicol (MIC = 100 μg/mL). This outcome pointed out the importance of fluorine substituent for antibacterial activity against *S. aureus*.

Compounds **1**, **2**, and **12** and chloramphenicol showed the same level of antibacterial activity against *E. faecalis* (ATCC 29212) with a MIC value of 200 μg/mL, whilst compounds **5** and **11** were more effective against *E. faecalis* than chloramphenicol. Generally electron withdrawing groups such as nitro and trifluoromethyl enhanced antibacterial activity, whereas electron donating substituents decreased antibacterial activity against *E. faecalis*.

In addition, *in vitro* antimycobacterial effects of the compounds were investigated against the drug resistant *M. tuberculosis* H37Rv strain using Microplate Alamar Blue Assay (MABA). Compounds **1**, **7**, **8**, **9**, **10**, **11**, and **12** exhibited antimycobacterial activity with a MIC value of 200 μg/mL, whereas other derivatives showed antimycobacterial activity with a MIC value of 400 μg/mL.

Table 3: Anticandidal activity of the thiosemicarbazone derivatives (MIC values in μg/mL).

Compound	A	B	C	D
1	400	400	200	200
2	400	200	200	200
3	400	400	400	200
4	400	400	200	200
5	400	200	200	200
6	400	400	200	200
7	400	400	200	200
8	400	400	200	200
9	400	400	200	200
10	400	400	200	200
11	400	400	400	200
12	400	400	400	200
Ketoconazole	200	200	3.125	50

A: *C. albicans* (ATCC 90028), B: *C. glabrata* (ATCC 90030), C: *C. krusei* (ATCC 6258), and D: *C. parapsilosis* (ATCC 22019).

As shown in Table 3, compounds **2** and **5** were the most potent antifungal derivatives against *C. glabrata* with a MIC value of 200 μg/mL when compared with ketoconazole (MIC = 200 μg/mL). It can be concluded that fluorine and nitro substituents increase antifungal activity against *C. glabrata*.

4. Conclusion

In the present paper, new thiosemicarbazone derivatives were synthesized and evaluated for their inhibitory effects on pathogenic bacteria including *M. tuberculosis* H37Rv. The antifungal effects of the compounds on *Candida* species were also investigated.

In general, the tested compounds exhibited more potent inhibitory effects on Gram-positive bacteria compared to Gram-negative bacteria. Among these derivatives, compound **2** was the most potent antibacterial derivative against *S. aureus* with a MIC value of 100 μg/mL when compared with chloramphenicol (MIC = 100 μg/mL). This outcome indicated that fluorine substituent increased antibacterial activity against *S. aureus*.

Compounds **5** and **11** exhibited the highest antibacterial activity against *E. faecalis* (ATCC 29212) with a MIC value of 100 μg/mL when compared with chloramphenicol (MIC = 200 μg/mL). This result demonstrated that nitro and trifluoromethyl groups increased antibacterial activity against *E. faecalis*.

According to MABA, the tested compounds exhibited weak to moderate antimycobacterial activity against *M. tuberculosis* H37Rv.

Compounds **2** and **5** and ketoconazole showed the same level of antifungal activity against *C. glabrata* with a MIC value of 200 μg/mL. This outcome pointed out the importance of fluorine and nitro substituents for antifungal activity against *C. glabrata*.

Conflict of Interests

The authors have declared no conflict of interests.

Acknowledgment

This study was supported by Anadolu University Scientific Research Projects Commission under Grants nos. 1404S137 and 1409S386.

References

[1] M. A. M. S. El-Sharief, S. Y. Abbas, K. A. M. El-Bayouki, and E. W. El-Gammal, "Synthesis of thiosemicarbazones derived from *N*-(4-hippuric acid)thiosemicarbazide and different carbonyl compounds as antimicrobial agents," *European Journal of Medicinal Chemistry*, vol. 67, pp. 263–268, 2013.

[2] F. C. Tenover, "Mechanisms of antimicrobial resistance in bacteria," *American Journal of Infection Control*, vol. 34, no. 5, supplement, pp. S3–S10, 2006.

[3] A. P. MacGowan, "Clinical implications of antimicrobial resistance for therapy," *Journal of Antimicrobial Chemotherapy*, vol. 62, no. 2, pp. ii105–ii114, 2008.

[4] M. Martinez and P. Silley, *Antimicrobial Drug Resistance*, Springer, Heidelberg, Germany, 2010.

[5] D. Armenise, M. Muraglia, M. A. Florio et al., "4*H*-1,4-benzothiazine, dihydro-1,4-benzothiazinones and 2-amino-5-fluorobenzenethiol derivatives: design, synthesis and *in vitro* antimicrobial screening," *Archiv der Pharmazie*, vol. 345, no. 5, pp. 407–416, 2012.

[6] N. Woodford and D. M. Livermore, "Infections caused by Gram-positive bacteria: a review of the global challenge," *Journal of Infection*, vol. 59, supplement 1, pp. S14–S16, 2009.

[7] S.-Y. Ruan and P.-R. Hsueh, "Invasive candidiasis: an overview from Taiwan," *Journal of the Formosan Medical Association*, vol. 108, no. 6, pp. 443–451, 2009.

[8] B. Moriyama, L. A. Gordon, M. McCarthy, S. A. Henning, T. J. Walsh, and S. R. Penzak, "Emerging drugs and vaccines for Candidemia," *Mycoses*, vol. 57, no. 12, pp. 718–733, 2014.

[9] M. K. Kathiravan, A. B. Salake, A. S. Chothe et al., "The biology and chemistry of antifungal agents: a review," *Bioorganic and Medicinal Chemistry*, vol. 20, no. 19, pp. 5678–5698, 2012.

[10] M. M. Canuto and F. G. Rodero, "Antifungal drug resistance to azoles and polyenes," *The Lancet Infectious Diseases*, vol. 2, no. 9, pp. 550–563, 2002.

[11] C. F. Rodrigues, S. Silva, and M. Henriques, "*Candida glabrata*: a review of its features and resistance," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 33, no. 5, pp. 673–688, 2014.

[12] C. G. Pierce and J. L. Lopez-Ribot, "Candidiasis drug discovery and development: new approaches targeting virulence for discovering and identifying new drugs," *Expert Opinion on Drug Discovery*, vol. 8, no. 9, pp. 1117–1126, 2013.

[13] C. G. Oliveira, P. I. D. S. Maia, P. C. Souza et al., "Manganese(II) complexes with thiosemicarbazones as potential anti-*Mycobacterium tuberculosis* agents," *Journal of Inorganic Biochemistry*, vol. 132, no. 1, pp. 21–29, 2014.

[14] L. Viganor, C. Skerry, M. McCann, and M. Devereux, "Tuberculosis: an inorganic medicinal chemistry perspective," *Current Medicinal Chemistry*, vol. 22, no. 18, pp. 2199–2224, 2015.

[15] S. H. Lee, "Diagnosis and treatment of latent tuberculosis infection," *Tuberculosis and Respiratory Diseases*, vol. 78, no. 2, pp. 56–63, 2015.

[16] E. Skoura, A. Zumla, and J. Bomanji, "Imaging in tuberculosis," *International Journal of Infectious Diseases*, vol. 32, pp. 87–93, 2015.

[17] M. J. Vorster, B. W. Allwood, A. H. Diacon, and C. F. Koegelenberg, "Tuberculous pleural effusions: advances and controversies," *Journal of Thoracic Disease*, vol. 7, no. 6, pp. 981–991, 2015.

[18] L. S. Meena, "An overview to understand the role of PE-PGRS family proteins in *Mycobacterium tuberculosis* H_{37}Rv and their potential as new drug targets," *Biotechnology and Applied Biochemistry*, vol. 62, no. 2, pp. 145–153, 2015.

[19] N. A. Mohamed, R. R. Mohamed, and R. S. Seoudi, "Synthesis and characterization of some novel antimicrobial thiosemicarbazone O-carboxymethyl chitosan derivatives," *International Journal of Biological Macromolecules*, vol. 63, pp. 163–169, 2014.

[20] M. M. Aly, Y. A. Mohamed, K. A. M. El-Bayouki, W. M. Basyouni, and S. Y. Abbas, "Synthesis of some new 4(3*H*)-quinazolinone-2-carboxaldehyde thiosemicarbazones and their metal complexes and a study on their anticonvulsant, analgesic, cytotoxic and antimicrobial activities—part-1," *European Journal of Medicinal Chemistry*, vol. 45, no. 8, pp. 3365–3373, 2010.

[21] U. Kulandaivelu, V. G. Padmini, K. Suneetha et al., "Synthesis, antimicrobial and anticancer activity of new thiosemicarbazone derivatives," *Archiv der Pharmazie*, vol. 344, no. 2, pp. 84–90, 2011.

[22] Y. Yu, D. S. Kalinowski, Z. Kovacevic et al., "Thiosemicarbazones from the old to new: iron chelators that are more than just ribonucleotide reductase inhibitors," *Journal of Medicinal Chemistry*, vol. 52, no. 17, pp. 5271–5294, 2009.

[23] R. K. Agarwal, L. Singh, and D. K. Sharma, "Synthesis, spectral, and biological properties of copper(II) complexes of thiosemicarbazones of *Schiff* bases derived from 4-aminoantipyrine and aromatic aldehydes," *Bioinorganic Chemistry and Applications*, vol. 2006, Article ID 59509, 10 pages, 2006.

[24] S. Arora, S. Agarwal, and S. Singhal, "Anticancer activities of thiosemicarbazides/thiosemicarbazones: a review," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 6, no. 9, pp. 34–41, 2014.

[25] N. Parul, N. Subhangkar, and M. Arun, "Antimicrobial activity of different thiosemicarbazone compounds against microbial pathogens," *International Research Journal of Pharmacy*, vol. 3, no. 5, pp. 351–363, 2012.

[26] N. E. Morrison and F. M. Collins, "Antimycobacterial activity of 2-acetylpyridine thiosemicarbazones in relation to their antileprosy activity," *International Journal of Leprosy*, vol. 49, no. 2, pp. 180–186, 1981.

[27] H. Beraldo and D. Gambino, "The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes," *Mini-Reviews in Medicinal Chemistry*, vol. 4, no. 1, pp. 31–39, 2004.

[28] J. A. Caminero, G. Sotgiu, A. Zumla, and G. B. Migliori, "Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis," *The Lancet Infectious Diseases*, vol. 10, no. 9, pp. 621–629, 2010.

[29] M. D. Altıntop, Ö. Atlı, S. Ilgın, R. Demirel, A. Özdemir, and Z. A. Kaplancıklı, "Synthesis and biological evaluation of new naphthalene substituted thiosemicarbazone derivatives as potent antifungal and anticancer agents," *European Journal of Medicinal Chemistry*, vol. 108, pp. 406–414, 2016.

[30] A. Özdemir, M. D. Altıntop, Z. A. Kaplancıklı, G. Turan-Zitouni, H. Karaca, and Y. Tunalı, "Synthesis and biological evaluation of pyrazoline derivatives bearing an indole moiety as new antimicrobial agents," *Archiv der Pharmazie*, vol. 346, no. 6, pp. 463–469, 2013.

[31] M. D. Altıntop, A. Özdemir, G. Turan-Zitouni et al., "Synthesis and biological evaluation of some hydrazone derivatives as new anticandidal and anticancer agents," *European Journal of Medicinal Chemistry*, vol. 58, pp. 299–307, 2012.