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Synthesis and Antimicrobial Activity of Some 3(2H)-Pyridazinone and 1(2H)-Phthalazinone Derivatives

Deniz S. DOĞRUER[1,*], Tijen ÖNKOL[1], Semiha ÖZKAN[2], Selda ÖZGEN[2], M. Fethi ŞAHİN[1]

[1] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara-TURKEY*
e-mail: denizdogruer2002@yahoo.com

[2] *Department of Microbiology, Faculty of Pharmacy, Gazi University, 06330 Etiler, Ankara-TURKEY*

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Some 3(2H)-pyridazinone and 1(2H)-phthalazinone derivatives were synthesized. The structures of these new compounds were confirmed by IR, ^{1}H-NMR, mass spectrum, and elemental analysis. The synthesized compounds were evaluated for their antibacterial activity against various gram-positive and gram-negative strains of bacteria and their clinical isolates and for their antimycobacterial activity against *M. tuberculosis* H37Rv. The results showed that the synthesized compounds were generally active against *B. subtilis* and its clinical isolate. Among the target compounds, compound **14c** exhibited the best antibacterial activity, with a MIC value of 15.62 μg/mL against *B. subtilis*. Compound **15e** had the highest antimycobacterial activity.

Key Words: 3(2H)-pyridazinone, 1(2H)-phthalazinone, antimicrobial activity

Introduction

Infectious diseases caused by bacteria have increased dramatically in recent years. In spite of many significant advances in antibacterial therapy, the widespread use and misuse of antibiotics have caused the emergence of bacterial resistance to antibiotics, which is a serious threat to public health. In particular, the emergence of multidrug resistant gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), and vancomycin-resistant Enterococci (VRE), has become a serious problem in the treatment of bacterial diseases.[1] Therefore, the development of new compounds to deal with resistant bacteria has become one of the most important areas of antibacterial research today.

*Corresponding author

In addition to the development of new and effective antibacterial agents against multidrug resistant gram-positive bacteria, recently attention has focused on the treatment of tuberculosis (TB). TB is a contagious disease infecting one-third of the world's population and killing between 2 and 3 million people each year. The increase in TB incidence during recent years is largely due to the AIDS epidemic and also the emergence of multidrug resistant tuberculosis (MDR-TB) strains. The treatment of MDR-TB has become a major concern worldwide.[2] Therefore, recent efforts have been directed toward exploring new, potent antimycobacterial agents with low toxicity profiles when compared with antimycobacterial agents currently on the market.

Many compounds carrying 3(2H)-pyridazinone and 1(2H)-phthalazinone rings are known to have different biological activities such as antiplatelet,[3] antihypertensive,[4] analgesic, and anti-inflammatory actions.[5–7] However, some compounds bearing 3(2H)-pyridazinone or 1(2H)-phthalazinone rings have been reported to have antimicrobial activity.[8–12] In addition, some benzenesulfonohydrazide derivatives have been reported to have antibacterial activity[13] (Figure 1).

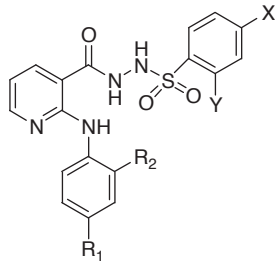


Figure 1. Benzenesulfonohydrazide derivatives with antibacterial activity in the literature.

On the basis of these findings, we synthesized new 3(2H)-pyridazinone and 1(2H)-phthalazinone derivatives carrying N′-(phenylsulfonyl)acetohydrazide moiety at position 2 of these rings in order to investigate their antibacterial and antimycobacterial activities (Figure 2).

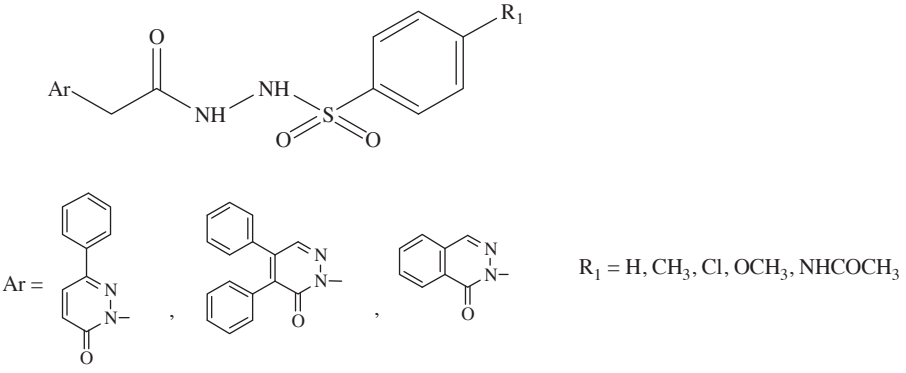


Figure 2. General structure of the synthesized compounds.

Experimental

Chemistry

All the chemicals used for the synthesis of the compounds were purchased from Aldrich Chemicals and Merck AG. Melting points of the compounds were recorded on an Electrothermal-9200 digital melting

points apparatus and are uncorrected. The IR spectra of the compounds were recorded on a Bruker Vector 22 IR (Opus Spectroscopic Software Version 2.0) spectrometer as KBr disks. The ^{1}H-NMR spectra were recorded with a Varian Mercury-400 FT-NMR spectrometer (Varian Inc., Palo Alto, CA, USA), in DMSO-d_6. The mass spectra were obtained on a Waters ZQ micromass LC-MS spectrometer (Waters Corporation, Milford, MA, USA) using the ESI(+) method. Elemental analysis was performed on a Leco 932 CHNS instrument (St. Joseph, MI, USA) and the results were within ± 0.4% of the theoretical values. Synthesis of compounds **1-5**,[17−20] **7** and **10**,[21,22] and **9** and **12**[7,23] was accomplished according to the previously reported procedures. Ethyl (4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetate **(8)** and 2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide **(11)** were prepared for the first time in this study.

Ethyl (4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetate (8)

4,5-Diphenyl-3(2H)-pyridazinone (0.01 mol), ethyl bromoacetate (0.015 mol), and anhydrous potassium carbonate (0.01 mol) in 20 mL of anhydrous DMF were stirred at room temperature for 2 h. The reaction mixture was poured into ice water and extracted with chloroform from the aqueous layer. The combined organic layer was dried over anhydrous Na_2SO_4 and the solvent was removed to dryness under reduced pressure and an oily residue was obtained. This compound was used to synthesize 2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide without further purification.

2-(4,5-Dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (11)

Ethyl (4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetate (0.01 mol) was refluxed with hydrazine hydrate (0.02 mol) in ethanol (20 mL) for 3 h. At the end of this period, the reaction mixture was kept in a refrigerator for several hours. The precipitate was filtered, dried, and crystallized from ethanol/water. mp 83 °C. FT-IR (KBr), cm^{-1}: 1668 (C=O ring), 1633 (C=O chain). ^{1}H-NMR (DMSO-d_6)δ: 9.30 (1H, s, N-H), 8.02 (1H, s, H^6), 7.28-7.09 (10H, m, phenyl protons), 5.04 and 4.69 (2H, s and s, -CH_2), 4.27 (2H, d, N-H).

General procedure for synthesis of 13a-13e, 14a-14e, 15a-15e

Substituted benzenesulfonyl chlorides (0.001 mol) were added to the solution of 2-(6-phenyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide or 2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide or 2-(1-oxo-2H-phthalazin-2-yl)acetohydrazide (0.001 mol) in pyridine (10 mL) at 0 °C. The resulting mixture was stirred at room temperature for 5 h. At the end of this period, the reaction mixture was poured into ice water. The precipitate was filtered, dried, and crystallized from an appropriate solvent.

N′-(Phenylsulfonyl)-2-(6-phenyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (13a)

Recrystallized from butanol to yield 80%. mp 228 °C. FT-IR (KBr), cm^{-1}: 1696 (C=O ring), 1660 (C=O chain), 1347, 1170 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.44, 10.22, 10.04, 9.81 (2H, 4 broad singlets, N-H), 8.09-8.04 (1H, dd, H^5), 7.87-7.45 (10H, m, 6-phenyl and phenyl protons), 7.09-7.04 (1H, dd, H^4), 5.04 and 4.70 (2H, s and s, -CH_2). MS ESI(+) m/e 385 (M+H, 100). Anal. ($C_{18}H_{16}N_4O_4S$): C, H, N, S calc. 56.24, 4.20, 14.57, 8.34 found 56.15, 4.06, 14.50, 8.42.

N′-[(4-Methylphenyl)sulfonyl]-2-(6-phenyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (13b)

Recrystallized from butanol to yield 78%. mp 178 °C. FT-IR (KBr), cm^{-1}: 1700 (C=O ring), 1668 (C=O chain), 1333, 1169 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.39, 9.92, 9.75 (2H, 3 broad singlets, N-H), 8.08-8.03 (1H, dd, H^5), 7.85-7.67 (4H, m, 6-phenyl $H^{2,6}$, phenyl $H^{2,6}$), 7.53-7.45 (3H, m, 6-phenyl $H^{3,4,5}$), 7.28 (2H, d, phenyl $H^{3,5}$), 7.08-7.04 (1H, dd, H^4), 5 and 4.69 (2H, s and s, -CH_2), 2.41 and 2.28 (3H, s and s, -CH_3). MS ESI(+) *m/e* 399 (M+H,100). Anal. ($C_{19}H_{18}N_4O_4S$): C, H, N, S calc. 57.27, 4.55, 14.06, 8.05 found 57.08, 4.28, 14.06, 8.23.

N′-[(4-Chlorophenyl)sulfonyl]-2-(6-phenyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (13c)

Recrystallized from butanol to yield 75%. mp 210 °C. FT-IR (KBr), cm^{-1}: 1698 (C=O ring), 1669 (C=O chain), 1344, 1174 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.49, 10.16, 9.78 (2H, 3 broad singlets, N-H), 8.07-8.02 (1H, dd, H^5), 7.84-7.43 (9H, m, 6-phenyl and phenyl protons), 7.07-7.03 (1H, dd, H^4), 5.02 and 4.68 (2H, s and s, -CH_2). MS ESI(+) *m/e* 419 (M+H, 100). Anal. ($C_{18}H_{15}ClN_4O_4S$): C, H, N, S calc. 51.62, 3.61, 13.38, 7.66 found 51.72, 3.37, 13.32, 7.74.

N′-[(4-Methoxyphenyl)sulfonyl]-2-(6-phenyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (13d)

Recrystallized from methanol to yield 78%. mp 192 °C. FT-IR (KBr), cm^{-1}: 1701 (C=O ring), 1669 (C=O chain), 1336, 1164 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.37, 9.81, 9.71 (2H, 3 broad singlets, N-H), 8.06-8.02 (1H, dd, H^5), 7.83-7.70 (4H, m, 6-phenyl $H^{2,6}$, phenyl $H^{2,6}$), 7.50-7.44 (3H, m, 6-phenyl $H^{3,4,5}$), 7.06-7.02 (1H, dd, H^4), 6.97 (2H, d, phenyl $H^{3,5}$), 4.97 and 4.67 (2H, s and s, -CH_2), 3.83 and 3.73 (3H, s and s, -CH_3). MS ESI(+) *m/e* 415 (M+H, 100). Anal. ($C_{19}H_{18}N_4O_5S$): C, H, N, S calc. 55.06, 4.38, 13.52, 7.74 found: 54.52, 4.55, 13.12, 7.62.

N′-[(4-Acetylaminophenyl)sulfonyl]-2-(6-phenyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (13e)

Recrystallized from butanol to yield 75%. mp 208 °C. FT-IR (KBr), cm^{-1}: 1701 (C=O ring), 1663 acetyl, 1655 (C=O chain), 1331, 1171 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.40, 10.37, 10.27, 10.01, 9.86, 9.73 (3H, 6 broad singlets, N-H), 8.06-8.02 (1H, dd, H^5), 7.83-7.66 (6H, m, 6-phenyl $H^{2,6}$ and phenyl protons), 7.49-7.43 (3H, m, 6-phenyl $H^{3,4,5}$), 7.06-7.01 (1H, dd, H^4), 4.97 and 4.67 (2H, s and s, -CH_2), 2.07 and 2.03 (3H, s and s, -CH_3). MS ESI(+) *m/e* 442 (M+H, 100). Anal. ($C_{20}H_{19}N_5O_5S$): C, H, N, S calc. 54.41, 4.34, 15.86, 7.26 found 53.98, 4.41, 15.34, 7.13.

N′-(Phenylsulfonyl)-2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (14a)

Recrystallized from ethyl acetate/hexane to yield 45%. mp 153 °C. FT-IR (KBr), cm^{-1}: 1673 (C=O ring), 1642 (C=O chain), 1350, 1170 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.44, 10.21, 10.04, 9.80 (2H, 4 broad singlets, N-H), 8.06 and 8.02 (1H, s and s, H^6), 7.86-7.49 (5H, m, phenyl protons), 7.31-7.01 (10H, m, 4,5-diphenyl protons), 5.07 and 4.68 (2H, s and s, -CH_2). MS ESI(+) *m/e* 461 (M+H, 100). Anal. ($C_{24}H_{20}N_4O_4S$): C, H, N, S calc. 62.60, 4.38, 12.17, 6.96 found 62.14, 4.61, 12.03, 6.95.

N′-[(4-Methylphenyl)sulfonyl]-2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (14b)

Recrystallized from ethyl acetate/hexane to yield 42.5%. mp 125 °C. FT-IR (KBr), cm^{-1}: 1700 (C=O ring), 1629 (C=O chain), 1344, 1166 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.40, 9.93, 9.72 (2H, 3 broad singlets,

N-H), 8.06 and 8.03 (1H, s and s, H^6), 7.70 (2H, d, phenyl $H^{2,6}$) 7.30-7.10 (12H, m, 4,5-diphenyl protons and phenyl $H^{3,5}$), 5.06 and 4.68 (2H, s and s, -CH_2), 2.42 and 2.35 (3H, s and s, -CH_3). MS ESI(+) *m/e* 475 (M+H, 100). Anal. ($C_{25}H_{22}N_4O_4S$): C, H, N, S calc. 63.28, 4.67, 11.8, 6.76 found 62.97, 4.45, 11.63, 6.77.

N′-[(4-Chlorophenyl)sulfonyl]-2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (14c)

Recrystallized from ethyl acetate/hexane to yield 38%. mp 114 °C. FT-IR (KBr), cm^{-1}: 1701 (C=O ring), 1629 (C=O chain), 1346, 1168 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.54, 10.29, 10.19, 9.78 (2H, 4 broad singlets, N-H), 8.07 and 8.04 (1H, s and s, H^6), 7.81 (2H, d, phenyl $H^{2,6}$), 7.53 (2H, d, phenyl $H^{3,5}$), 7.31-7.10 (10H, m, 4,5-diphenyl protons), 5.09 and 4.69 (2H, s and s, -CH_2). MS ESI(+) *m/e* 495 (M+H, 100). Anal. ($C_{24}H_{19}ClN_4O_4S$): C, H, N, S calc. 58.24, 3.87, 11.32, 6.48 found 58.01, 3.83, 11.28, 6.56.

N′-[(4-Methoxyphenyl)sulfonyl]-2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (14d)

Recrystallized from ethyl acetate/hexane to yield 42%. mp 170 °C. FT-IR (KBr), cm^{-1}: 1700 (C=O ring), 1624 (C=O chain), 1345, 1160 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.38, 9.99, 9.83, 9.71 (2H, 4 broad singlets, N-H), 8.05 and 8.02 (1H, s and s, H^6), 7.73 (2H, d, phenyl $H^{2,6}$) 7.30-7.0.8 (10H, m, 4,5-diphenyl protons), 7.01 (2H, d, phenyl $H^{3,5}$), 5.06 and 4.68 (2H, s and s, -CH_2), 3.85 and 3.78 (3H, s and s, -CH_3). MS ESI(+) *m/e* 491 (M+H, 100). Anal. ($C_{25}H_{22}N_4O_5S$) C, H, N, S calc. 61.21, 4.52, 11.42, 6.54 found 61.04, 4.56, 11.15, 6.52.

N′-[(4-Acetylaminophenyl)sulfonyl]-2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide (14e)

Recrystallized from butanol yield 40%. mp 275 °C. FT-IR (KBr), cm^{-1}: 1700 (C=O ring), 1669 acetyl, 1629 (C=O chain), 1350, 1166 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.39, 10.38, 10.30, 10.01, 9.87, 9.71 (3H, 6 broad singlets, N-H), 8.03 and 7.99 (1H, s and s, H^6), 7.78-7.73 (4H, m, phenyl protons) 7.28-7.06 (10H, m, 4,5-diphenyl protons), 5.04 and 4.65 (2H, s and s, -CH_2), 2.08 and 2.05 (3H, s and s, -CH_3). MS ESI(+) *m/e* 518 (M+H, 100). Anal. ($C_{26}H_{23}N_5O_5S$): C, H, N, S calc. 60.34, 4.48, 13.53; 6.20 found 60.57, 4.46, 13.39, 6.07.

N′-(Phenylsulfonyl)-2-(1-oxo-2H-phthalazin-2-yl)acetohydrazide (15a)

Recrystallized from methanol to yield 87%. mp 219 °C. FT-IR (KBr), cm^{-1}: 1710 (C=O ring), 1637 (C=O chain), 1341, 1173 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.41, 10, 9.75 (2H, 3 broad singlets, N-H), 8.43 and 8.40 (1H, s and s, H^4), 8.23 (1H, d, H^8), 7.98-7.51 (8H, m, $H^{5,6,7}$, phenyl protons), 5.07 and 4.65 (2H, s and s, -CH_2). MS ESI(+) *m/e* 359 (M+H, 100). Anal. ($C_{16}H_{14}N_4O_4S$): C, H, N, S calc. 53.62, 3.94, 15.63, 8.95. found 53.60, 3.93, 15.57, 9.08.

N′-[(Methylphenyl)sulfonyl]-2-(1-oxo-2H-phthalazin-2-yl)acetohydrazide (15b)

Recrystallized from methanol to yield 82.5%. mp 185 °C. FT-IR (KBr), cm^{-1}: 1711 (C=O ring), 1641 (C=O chain), 1339, 1172 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.35, 9.89, 9.67 (2H, 3 broad singlets, N-H), 8.43 and 8.40 (1H, s and s, H^4), 8.23 (1H, d, H^8), 7.97-7.86 (3H, m, $H^{5,6,7}$), 7.70 (2H, d, pheny $H^{2,6}$), 7.32 (2H, d, pheny $H^{3,5}$), 5.06 and 4.66 (2H, s and s, -CH_2), 2.42 and 2.33 (3H, s and s, -CH_3). MS ESI(+) *m/e* 373 (M+H, 100). Anal. ($C_{17}H_{16}N_4O_4S$): C, H, N, S calc. 54.83, 4.33, 15.04, 8.61 found 54.53, 4.28, 14.95, 8.73.

N′-[(Chlorophenyl)sulfonyl]-2-(1-oxo-2H-phthalazin-2-yl)acetohydrazide (15c)

Recrystallized from methanol to yield 79.5%. mp 222 °C. FT-IR (KBr), cm^{-1}: 1708 (C=O ring), 1639 (C=O chain), 1344, 1176 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.46, 10.18, 9.58 (2H, 3 broad singlets, N-H), 8.43 and 8.41 (1H, s and s, H^4), 8.24 (1H, d, H^8), 7.97-7.86 (3H, m, $H^{5,6,7}$), 7.82 (2H, d, pheny-$H^{2,6}$), 7.59 (2H, d, pheny-$H^{3,5}$), 5.06 and 4.67 (2H, s and s, -CH_2). MS ESI(+) m/e 393 (M+H, 100). Anal. ($C_{16}H_{13}ClN_4O_4S$): C, H, N, S calc. 48.92, 3.34, 14.26, 8.16 found 48.73, 3.31, 14.24, 8.35.

N′-[(Methoxyphenyl)sulfonyl]-2-(1-oxo-2H-phthalazin-2-yl)acetohydrazide (15d)

Recrystallized from methanol to yield 85%. mp 226 °C. FT-IR (KBr), cm^{-1}: 1697 (C=O ring), 1640 (C=O chain), 1342, 1168 (SO_2). ^{1}H-NMR (DMSO-d_6)δ: 10.37, 9.80, 9.67 (2H, 3 broad singlets, N-H), 8.43 and 8.41 (1H, s and s, H^4), 8.24 (1H, d, H^8), 7.97-7.85 (3H, m, $H^{5,6,7}$), 7.75 (2H, d, pheny-$H^{2,6}$), 7.03 (2H, d, pheny-$H^{3,5}$), 5.06 and 4.67 (2H, s and s, -CH_2), 3.87 and 3.78 (3H, s and s, -CH_3). MS ESI(+) m/e 389 (M+H, 100). Anal. ($C_{17}H_{16}N_4O_5S$): C, H, N, S calc. 52.57, 4.15, 14.43, 8.26. found 52.49, 4.20, 14.38, 8.41.

N′-[(Acetylaminophenyl)sulfonyl]-2-(1-oxo-2H-phthalazin-2-yl)acetohydrazide (15e)

Recrystallized from butanol to yield 77%. mp 273 °C. FT-IR (KBr), cm^{-1}: 1708 (C=O ring), 1676 acetyl, 1637 (C=O chain), 1347, 1170 (SO_2). ^{1}H-NMR (DMSO-d_6) δ: 10.41, 10.35, 10.29, 9.84, 9.67 (3H, 5 broad singlets, N-H), 8.43 and 8.40 (1H, s and s, H^4), 8.22 (1H, d, H^8), 7.97-7.69 (7H, m, $H^{5,6,7}$, phenyl protons), 5.06 and 4.66 (2H, s and s, -CH_2), 2.10 and 2.07 (3H, s and s, -CH_3). MS ESI(+) m/e 416 (M+H, 100). Anal. ($C_{18}H_{17}N_5O_5S$): C, H, N, S calc. 52.04, 4.12, 16.86, 7.72 found 51.98, 4.29, 16.58, 7.75.

Antibacterial activity[14]

Minimum inhibitory concentration (MIC) values of the synthesized compounds were determined by broth microdilution.[14] Four gram-positive (*S. aureus* ATCC 29213 and methicillin-resistant *S. aureus* (MRSA, clinical isolate), *B. subtilis* ATCC 6633, and *B. subtilis* clinical isolate) and 4 gram-negative (*E. coli* ATCC 25922 and *E. coli* producing extended spectrum β-lactamase (ESBL, clinical isolate), *P. aeruginosa* ATCC 27853, and *P. aeruginosa* clinical isolate) bacteria were used. Sulfanilamide, sulfamethoxazole, and ampicillin were used as references. The synthesized compounds and references were dissolved in DMSO/H_2O (50%), at a concentration of 500 μg/mL. Twofold dilutions of the synthesized compounds and reference compounds were prepared (250, 125,.....0.24 μg/mL). All bacteria were cultivated in Mueller-Hinton agar (Merck). The final inoculum concentration was 10^4 cfu/mL for bacteria in the wells after inoculations. The microplates were incubated at 37 °C and read visually after 24 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 had no visible turbidity. DMSO concentration in the final solutions was not above 12.5% for antibacterial activity.

Antimycobacterial activity[15,16]

The synthesized compounds were evaluated for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (ATCC 27294) using proportion agar dilution according to CLSI (formerly NCCLS).[15,16] Isoniazid and rifampicin were used as control agents. H37Rv is susceptible to all the primary and secondary antituberculosis drugs. The stock solution of the synthesized compounds and reference compounds was prepared

in DMSO/H_2O (50%), at a concentration of 1000 μg/mL. These solutions were filtered through a 0.22 μm filter (Millipore, USA). Middlebrook 7H11 agar was kept in a water bath at 50-56 °C and supplemented with 10% oleic acid albumin dextrose catalase (OADC). The synthesized compounds and references were added to obtain an appropriate final concentration in the medium. The final concentrations of INH were 0.2 and 1 μg/mL and the final concentration of rifampicin was 1 μg/mL. The synthesized compounds were prepared at concentrations of 5, 10, 15, and 20 μg/mL. Since the synthesized compounds were not active on *M. tuberculosis* at concentrations of 5, 10, or 15 μg/mL, they were only evaluated at a concentration of 20 μg/mL. Agar without any references and synthesized compounds were used as a positive growth control. The prepared medium was dispensed into sterile plastic petri plates in 5 mL amounts and the plates were dried in a laminar-flow hood. DMSO concentration in the final solutions was not above 1% for antimycobacterial activity.

Inoculum preparation

H37Rv cultivated in Middlebrook 7H11 agar was suspended with Middlebrook 7H9 broth supplemented with 10% OADC and 0.05% Tween 80. Tubes containing 6 to 10 glass beads and 5 mL of Middlebrook 7H9 broth suspended with H37Rv were homogenized by vortex. The turbidity of the supernatant suspension was adjusted to McFarland 1 standard (contains approximately 10^7 cfu/mL) by adding Middlebrook 7H9 broth. The standardized suspensions were diluted 4-fold with sterile saline and 0.1 mL of the diluted suspensions was inoculated onto the control plates (blood agar and agar without any references and synthesized compounds) and onto each plate containing the synthesized compounds and references. The plates were incubated at 37 °C in an atmosphere of 5% CO_2 for 3 weeks.

Reading the plates

The first week the blood agar control plate was examined in terms of bacterial contamination.

Plates having references and the synthesized compounds were examined every week in terms of bacteria growth.

At the end of 3 weeks, all plates were evaluated by counting the number of colonies growing on plates.

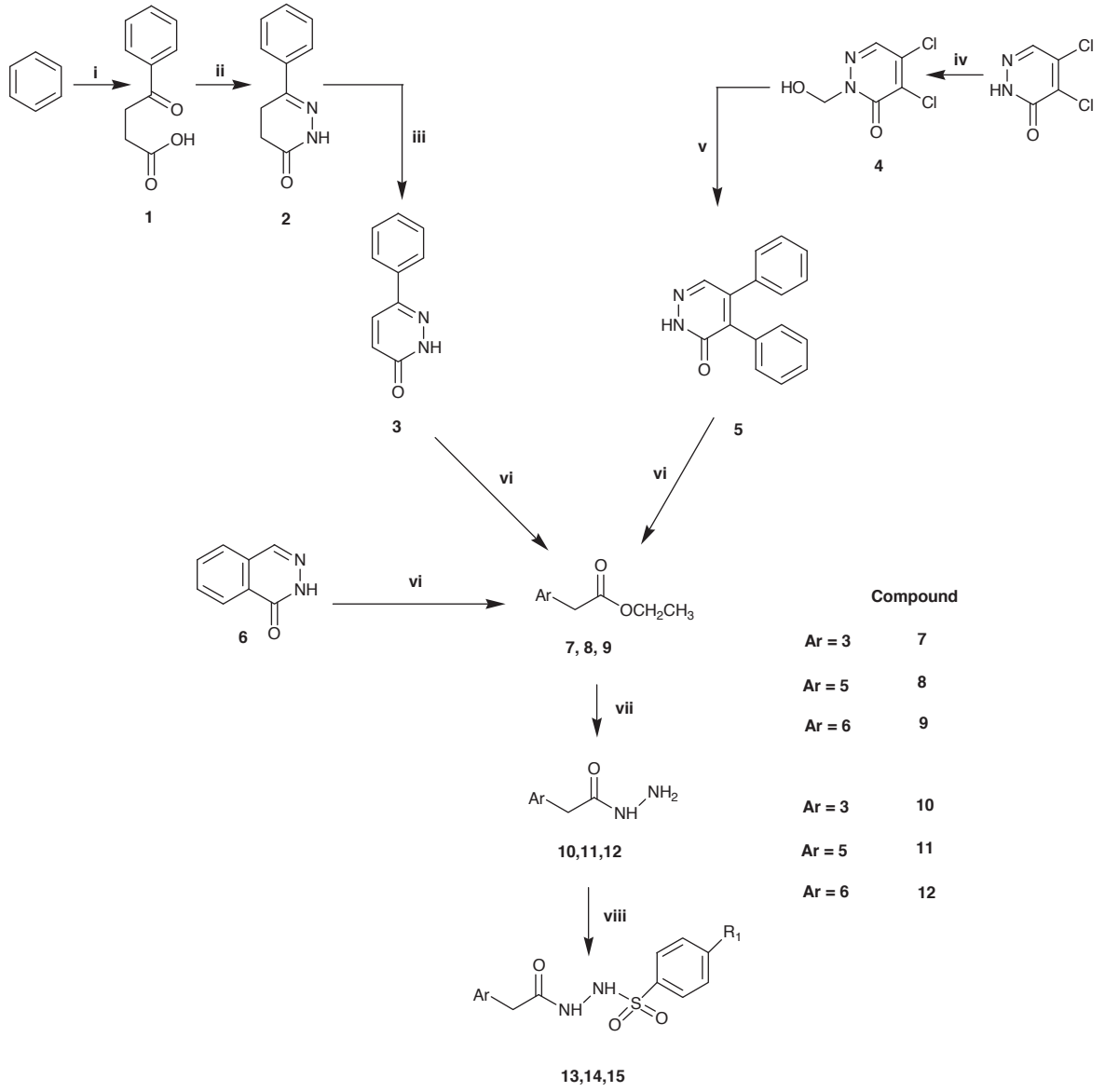
Percent resistance was calculated using the following formula for references and the synthesized compounds:

$$\text{Resistance } \% = \frac{\text{The number of colonies on plates having references and the synthesized compounds}}{\text{The number of colonies on control plate}} \times 100$$

Results and Discussion

Chemistry

Fifteen new target compounds were synthesized by the reaction of substituted benzenesulfonyl chloride with 2-(6-phenyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide or 2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide or 2-(1-oxo-2H-phthalazin-2-yl)acetohydrazide in pyridine. Structures of the synthesized compounds were elucidated by IR, ^{1}H-NMR, mass spectrum, and elemental analysis.



Ar = 3	R_1 = H **(13a)**, CH_3 **(13b)**, Cl **(13c)**, OCH_3 **(13d)**, $NHCOCH_3$ **(13e)**
Ar = 5	R_1 = H **(14a)**, CH_3 **(14b)**, C l **(14c)**, OCH_3 **(14d)**, $NHCOCH_3$ **(14e)**
Ar = 6	R_1 = H **(15a)**, CH_3 **(15b)**, Cl **(15c)**, OCH_3 **(15d)**, $NHCOCH_3$ **(15e)**

i = succinic anhydride, $AlCl_3$; ii = NH_2NH_2/C_2H_5OH ; iii =Br_2/AcOH ; iv = CH_2O; v = $PhB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3 , Toluene/H_2O, vi = ethyl bromoacetate/K_2CO_3/DMF; vii = NH_2NH_2/C_2H_5OH; viii =p-substituted benzenesulfonyl chlorides/pyridine

Scheme. Synthetic route of the synthesized compounds.

Synthetic route of the synthesized compounds is given in the Scheme. Benzene, 4,5-dichloro-3(2H)-pyridazinone, and 1(2H)-phthalazinone were used as the starting materials. Synthesis of compounds **1-5,**[17−20] **7** and **10,**[21,22] and **9** and **12**[7,23] was accomplished according to the previously reported procedures. Ethyl (4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetate **(8)** and 2-(4,5-dipheyl-3-oxo-2H-pyridazin-2-yl)acetohydrazide **(11)** were prepared for first time in this study. However, since the ester derivative of

4,5-dipheyl-3(2H)-pyridazinone **(8)** was obtained as an oily residue, it was used to synthesize the hydrazide derivative of 4,5-dipheyl-3(2H)-pyridazinone **(11)** without any further purification.

Based on ^{1}H-NMR spectra of the target compounds and the literature,[24] we suggested that the target compounds had rotamers due to hydrogen bonding between N-H next to carbonyl at the side chain and the carbonyl group of pyridazinone or phthalazinone rings. That is, the target compounds had rotamers, one of which was with hydrogen bonding and the other without hydrogen bonding. Therefore, CH_2 protons at the side chain and the protons belonging to methyl, methoxy, and acetylamino groups at the para position on the phenyl ring gave 2 separate singlets. In addition, some of aromatic protons that were expected to appear as 1 singlet gave 2 separate singlets. For example, the proton at position 6 of the 4,5-dipheyl-3(2H)-pyridazinone ring and the proton at position 4 of 1(2H)-phthalazinone gave 2 separate singlets. Likewise, the protons at both positions 4 and 5 of the 6-phenyl-3(2H)-pyridazinone ring gave 2 distinct doublets on top of each other instead of a doublet. According to the rotamerism mentioned above, N-H groups also showed separate signals more than expected, around 10 ppm. One might say that the proportion of rotamerism is 1:3 (low field/high field) depending on the integral values of each singlet pair.

Antibacterial and Antimycobacterial Activity

The synthesized compounds were tested in vitro for antibacterial activity against gram-positive *S. aureus*, methicillin-resistant *S. aureus* (MRSA, clinical isolate), *B. subtilis*, *B. subtilis* (clinical isolate), gram-negative *E. coli* and *E. coli* producing extended spectrum β-lactamase (ESBL, clinical isolate), *P. aeruginosa*, and *P. aeruginosa* (clinical isolate) bacteria using broth microdilution and for antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv using proportion agar dilution according to CLSI (formerly NCCLS). Sulfanilamide, sulfamethoxazole, ampicillin, isoniazid, and rifampicin were used as references.

As shown in Table 1, none of the target compounds had activity against gram-negative bacteria. The target compounds were generally active against *B. subtilis* and its clinical isolate. When the chemical structures of the active compounds were taken into consideration, it was determined that 1(2H)-phthalazinone **(15a-15e)** and 4,5-dipheyl-3(2H)-pyridazinone derivatives **(14a-14e)** were more active than 6-phenyl-3(2H)-pyridazinone derivatives **(13a-13e)** against *B. subtilis* and its clinical isolate. While compounds **14b, 14d, 14e, 15a, 15c,** and **15e** were as active as sulfanilamide against *B. subtilis* clinical isolate, compounds **13d** and **15b** were as active as sulfanilamide against both *B. subtilis* and its clinical isolate. Compounds **14a** and **14c** were 2 times as active as sulfanilamide against *B. subtilis* clinical isolate and their antibacterial activity was 50% of that of sulfamethoxazole against *B. subtilis* clinical isolate. Compound **15a** was 2 times as active as sulfanilamide against *B. subtilis* and its activity was equal to that of sulfamethoxazole against *B. subtilis*. Compound **15d** was as active as sulfamethoxazole against *B. subtilis* and its antibacterial activity was 50% of that of sulfamethoxazole against *B. subtilis* clinical isolate. Moreover, this compound was 2 times as active as sulfanilamide against both *B. subtilis* and its clinical isolate. Among the target compounds, compound **14c** exhibited the best antibacterial activity, with a MIC value of 15.62 μg/mL, against *B. subtilis.* Moreover, this derivative was the only compound that was as active as ampicillin, with a MIC value of 31.25 μg/mL, against *S. aureus.*

As shown in Table 2, even if the target compounds did not seem to have antimycobacterial activity in comparison with antimycobacterial references, compound **15e** had the highest antimycobacterial activity. In addition, compounds **13a**, **13c**, and **15c** exhibited antimycobacterial activity comparable to that of compound **15e.**

Table 1. Minimum inhibitory concentrations (MICs, μg/mL) of the synthesized compounds.

Compound	A	B	C	D	E	F	G	H
13a	250	125	250	250	250	250	250	250
13b	250	125	125	250	250	250	250	250
13c	250	125	125	250	250	125	125	250
13d	250	250	**62.5**	**62.5**	250	250	250	250
13e	250	125	125	125	250	125	250	250
14a	250	125	125	**31.25**	250	250	125	125
14b	250	250	125	**62.5**	250	250	250	125
14c	**31.25**	125	**15.62**	**31.25**	250	250	250	125
14d	250	125	125	**62.5**	250	250	250	125
14e	250	125	125	**62.5**	125	250	250	125
15a	250	125	**31.25**	**62.5**	250	250	250	250
15b	250	125	**62.5**	**62.5**	250	250	250	250
15c	250	125	125	**62.5**	250	250	250	250
15d	250	125	**31.25**	**31.25**	250	250	250	250
15e	250	125	250	**62.5**	125	125	250	250
Sulfanilamide	125	125	62.5	62.5	62.5	62.5	125	125
Sulfamethoxazole	62.5	15.62	31.25	15.62	15.62	31.25	62.5	62.5
Ampicillin	31.25	62.5	0.48	0.48	7.81	62.5	125	125

A: *S. aureus* ATCC 29213, B: MRSA clinical isolate, C: *B. subtilis* ATCC 6633, D: *B. subtilis* clinical isolate, E: *E. coli* ATCC 25922, F: *E. coli* clinical isolate, G: *P. aeruginosa* ATCC 27853,
H: *P. aeruginosa* clinical isolate

Table 2. Antimycobacterial activity results of the synthesized compounds.

Compound	Concentration μg/mL*	The number of colonies	Resistance %
13a	20	78	**52.70**
13b	20	93	62.83
13c	20	70	**47.29**
13d	20	122	82.43
13e	20	137	92.56
14a	20	90	60.81
14b	20	145	97.97
14c	20	107	72.29
14d	20	96	64.86
14e	20	120	81.08
15a	20	110	74.32
15b	20	112	75.67
15c	20	71	**47.97**
15d	20	110	74.32
15e	20	64	**43.24**
INH	0.2	no growth	-
INH	1	no growth	-
Rifampicin	1	no growth	-
(+) Control		**148**	

*The synthesized compounds are not active on *M. tuberculosis* at concentrations of 5, 10, or 15 μg/mL.

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