Synthesis of linezolid-like molecules and evaluation of their antimicrobial activities

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3-Fluoro-4-(morpholin-4-yl)aniline (2), prepared from 3,4-difluoronitrobenzene, was converted to the corresponding Schiff base (3) by treatment with indol-3-carbaldehyde. The treatment of thiourea 4 and carbothioamide derivatives 9 with ethyl bromoacetate or 4-substituted phenacyl bromides generated the corresponding thiazolidinone (5 and 13) and thiazoline (6 and 12) derivatives, respectively. The acidic or basic treatment of carbothioamide 9 produced 1,3,4-thiadiazole (11) or 1,2,4-triazole (10) compounds, respectively. The structural assignments of the new compounds were based on elemental analysis and spectral (IR, ¹H-NMR, ¹³C-NMR, and LC-MS) data. The antimicrobial activity study revealed that all compounds showed good antitubercular activities.

Key Words: 1,3-Thiazole, 1,3,4-thiadiazole, 1,2,4-triazole, linezolid, antimicrobial activity

Introduction

Despite the existence of a number of antibiotics that are used for the treatment of bacterial infections, the mortality and morbidity caused by gram-positive bacteria have an alarming worldwide impact on the human population due to the increasing number of multidrug-resistant microbial pathogens. Mycobacterium tuberculosis is the etiological agent for tuberculosis (TB). The incidence of TB, which causes approximately 3 million deaths worldwide every year, has steadily risen in the past years, and it is the world’s second most common cause of

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death from infectious disease, after acquired immunodeficiency syndrome (AIDS). Incidences of TB and AIDS occurring together are common. According to the World Health Organization, about 30 million people will be infected within the next 20 years. Thus, the exploration of a new class of antibacterial agents with novel mechanisms is crucial to combat multidrug-resistant infections.1–5

Oxazolidinones are a relatively new class of synthetic antibacterial agents, having a new mechanism of action that involves early inhibition of bacterial protein synthesis. This class of compounds is particularly active against gram-positive organisms. Oxazolidinones are thought to not be cross-resistant with other types of antibiotics due to their different action mechanism, which includes interaction with the bacterial ribosome to inhibit bacteria.6–11 Important examples of this class are the morpholine derivative linezolid and the piperazine derivative eperezolid (Scheme). The thiomorpholine analog of linezolid, PNU-100480, has also been reported to exhibit antimicrobial activity. The compounds, including an azole moiety instead of the morpholine nucleus in the linezolid structure, have also been reported as antimicrobial agents.12

Thiazolidinone derivatives have been further reported to possess diverse pharmacological properties, such as antibacterial, antifungal, anticonvulsant, anticancer, antituberculosis, and anti-human immunodeficiency virus type 1 (HIV-1) activities. Thiazolidinones are novel inhibitors of the bacterial enzyme MurB, a precursor acting during the biosynthesis of peptidoglycan as an essential component of the cell wall of both gram-positive and gram-negative bacteria.13–21

The identification and synthesis of combinational chemotherapeutic drugs with different mechanisms of action and with few side effects is an important part of the efforts to overcome antimicrobial resistance.22 A
recent survey of novel small-molecule therapeutics has revealed that the majority result from an analog-based approach and that their market value represents two-thirds of all drug sales.²³

**Experimental**

**Chemistry**

All chemicals were purchased from Fluka Chemie AG (Buchs, Switzerland) and were used without further purification. Melting points of the synthesized compounds were determined in open capillaries on a Büchi B-540 melting point apparatus (Büchi Labortechnik AG, Flawil, Switzerland) and are uncorrected. Reactions were monitored by thin-layer chromatography (TLC) on silica gel 60 F254 aluminum sheets. The mobile phase was ethanol and ethyl acetate (1:1), and detection was done with UV light. Infrared (IR) spectra were recorded as potassium bromide pellets using a PerkinElmer 1600 series Fourier transform infrared (FTIR) spectrometer (Waltham, MA, USA). ¹H-NMR and ¹³C-NMR spectra were recorded on a Bruker AVANCE II 400-MHz NMR spectrometer (Bruker Corporation, Billerica, MA, USA), with chemical shift in ppm downfield from TMS as an internal reference. The elemental analysis was performed on a Costech Elemental Combustion System CHNS-O elemental analyzer (Costech Analytical Technologies, Valencia, CA, USA). All compounds gave C, H, and N analysis results within ±0.4% of the theoretical values. The mass spectra were obtained on a Quattro liquid chromatography-mass spectrometry (LC-MS) (70 eV) instrument (Waters Corporation, Milford, MA, USA).

**Compounds 1 and 2 are commercially available.**

**4-(2-Fluoro-4-nitrophenyl)morpholine (1):** 3,4-Difluoronitrobenzene (10 mmol) was refluxed with an excess amount of morpholine (20 mL) for 8 h (TLC-controlled). The mixture was then poured into ice water. The precipitated product was filtered off and recrystallized from ethanol (yield: 2.20 g, 97%). Mp 111°C (yellow crystals); IR (KBr) cm⁻¹: 3074 (aromatic CH), 1345 and 1519 (-NO₂); ¹H-NMR (DMSO-d₆): δ ppm 3.23 (brs, 4H, 2CH₂), 3.70 (brs, 4H, 2CH₂), 7.09 (t, 1H, arH, J = 9.2 Hz), 7.94 (d, 2H, arH, J = 7.8 Hz); ¹³C-NMR (DMSO-d₆): δ ppm 50.06-50.16 (2CH₂), arC [112.61 (CH), 118.37 (CH), 121.92 (CH), 140.15 (C), 145.87 (C), 150.31 (C)]; MS m/z (%): 136.83 (77), 148.78 (24), 151.84 (18), 165.80 (20), 179.88 (42), 180.82 (48), 182.82 (100), 209.85 (62), 226.94 ([M]⁺, 36); Anal. Calcd. (%) for C₁₀H₁₁FN₂O₃: 53.10, C; 4.90, H; 12.38, N. Found: 52.70, C; 4.60, H; 12.00, N.

**3-Fluoro-4-(morpholin-4-yl)aniline (2):** Pd/C catalyst (5 mmol) was added to a solution of compound 1 (10 mmol) in butanol, and the mixture was allowed to reflux in the presence of hydrazine hydrate (50 mmol) for 6 h. The progress of the reaction was monitored by TLC. After completion of the reaction, the catalyst was removed by filtration, and the reaction solvent was removed under reduced pressure. The obtained white solid was recrystallized from ethanol to afford the desired compound (yield: 1.66 g, 85%). Mp 119°C (white crystals); IR (KBr) cm⁻¹: 3074 (aromatic CH), 1345 and 1519 (-NO₂); ¹H-NMR (DMSO-d₆): δ ppm 3.23 (brs, 4H, 2CH₂), 3.70 (brs, 4H, 2CH₂), 4.98 (s, 2H, NH₂, D₂O exchange), 6.26-6.36 (m, 2H, arH), 6.68-6.78 (m, 1H, arH); ¹³C-NMR (DMSO-d₆): δ ppm 50.06-50.16 (2CH₂), 66.50 (2CH₂), arC [101.90 (CH), 109.84 (CH), 120.63 (CH), 129.17 (C), 145.58 (C), 153.98 (C)]; MS m/z (%): 196.84 ([M]⁺, 100), 197.90 ([M+1]⁺, 11); Anal. Calcd. (%) for C₁₀H₁₃FN₂O: 53.10, C; 4.90, H; 12.38, N. Found: 61.46, C; 6.43, H; 14.18, N.

**3-Fluoro-N-[(1H-indol-3-yl)methylidene]-4-(morpholin-4-yl)aniline (3):** A solution of com-
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Compound 2 (10 mmol) in absolute ethanol was refluxed with indol-3-carbaldehyde (10 mmol) for 6 h. The reaction content was allowed to reach room temperature, and a solid appeared. This crude product was filtered off and recrystallized from acetone to obtain the desired compound (yield: 3.07 g, 95%). Mp 180 °C; IR (KBr) cm⁻¹: 3092 (aromatic CH), 1443 (C=N); 1H-NMR (DMSO-d₆): δ ppm 2.91 (s, 4H, 2CH₂), 3.71 (s, 4H, 2CH₂), 6.70-6.80 (brs, 5H, arH), 6.91-7.00 (t, 3H, arH, J = 9.1 Hz), 9.11 (s, 1H, N=CH); 13C-NMR (DMSO-d₆): δ ppm 51.64 (2CH₂), 66.84 (2CH₂), 107.04 (N=CH), arC [107.51 (CH), 115.22 (2CH), 120.80 (2CH), 120.88 (2CH), 130.33 (CH), 134.54 (C), 136.43 (C), 136.64 (C), 146.95 (C), 153.51 (C), 158.38 (C)]; MS m/z (%): 298.19 (91), 298.38 (86), 298.51 (54), 300.2 (36), 301.13 (26), 324.29 ([M+1]+, 40), 336.17 (60), 364.14 ([M+2+K]+, 61); Anal. Calcd. (%) for C₁₉H₁₈FN₃O: 70.57, C; 5.61, H; 12.99, N. Found: 70.20, C; 5.75, H; 12.65, N.

1-[3-Fluoro-4-(morpholin-4-yl)phenyl]-3-(4-fluorophenyl)thiourea (4): A solution of compound 2 (10 mmol) in absolute ethanol was stirred under reflux in the presence of 4-fluorophenyl isothiocyanate (10 mmol) for 7 h. The progress of the reaction was monitored by TLC. After the reaction mixture cooled to room temperature, a solid formed. This crude product was separated by filtration and recrystallized from ethanol to give the target compound (yield: 1.56 g, 67%). Mp 160 °C; IR (KBr) cm⁻¹: 3202 (2NH), 3022 (aromatic CH), 1219 (C=S); 1H-NMR (DMSO-d₆): δ ppm 2.95 (s, 4H, 2CH₂), 3.72 (s, 4H, 2CH₂), 6.93-7.19 (m, 4H, arH), 7.33-7.40 (m, 3H, arH), 9.73 (s, 2H, D₂O exchange); 13C-NMR (DMSO-d₆): δ ppm 51.31 (CH₂), 51.36 (CH₂), 66.86 (2CH₂), arC [112.61 (CH), 115.51 (CH), 119.23 (2CH), 120.72 (2CH), 126.92 (CH), 134.66 (2C), 137.08 (2C), 152.34 (C)], 180.10 (C=S); MS m/z (%): 101.85 (46), 103.10 (37), 105.73 (33), 109.80 (26), 121.25 (18), 152.91 (50), 196.02 (25), 196.96 (50), 228.99 (36), 264.97 (38), 300.07 (91), 301.07 (26), 316.09 (100), 317.09 (21), 350.06 ([M]+, 40); Anal. Calcd. (%) for C₁₇H₁₇F₂N₃OS: 58.44, C; 4.90, H; 12.03, N. Found: 58.10, C; 5.23, H; 11.81, N.

1-[3-Fluoro-4-(morpholin-4-yl)phenyl]-3-[3-(4-fluorophenyl)-4-oxo-1,3-thiazolidin-2-ylidene]thiourea (5): A mixture of compound 4 (10 mmol) and ethyl bromoacetate in absolute ethanol was allowed to reflux in the presence of dried sodium acetate (50 mmol) for 5 h; the progress of the reaction was monitored by TLC. After the reaction mixture cooled to room temperature, a solid formed. This crude product was separated by filtration and recrystallized from dimethyl sulfoxide to afford the desired product (yield: 2.44 g, 63%). Mp 206 °C; IR (KBr) cm⁻¹: 1725 (C=O), 1449 (C=N); 1H-NMR (DMSO-d₆): δ ppm 2.99-2.95 (brs, 4H, 2CH₂), 3.72 (s, 4H, 2CH₂), 4.12 (s, 2H, thiazole C₄), 6.68-6.74 (m, 3H, arH), 6.98-7.29 (m, 4H, arH); MS m/z (%): 117.87 (76), 117.99 (86), 148.71 (58), 148.84 (79), 152.91 (73), 153.09 (58), 154.72 (43), 155.10 (32), 214.79 (38), 214.91 (48), 229.05 (100), 229.24 (72), 230.87 (55), 270.16 (37), 271.16 (50), 273.10 (58), 284.18 (56), 300.20 (35), 370.27 (33), 371.34 (75), 383.29 (78), 384.36 (46), 413.39 ([M+1+Na]+, 60); Anal. Calcd. (%) for C₁₉H₁₇F₂N₃O₂S: 58.60, C; 4.40, H; 10.79, N. Found: 58.77, C; 4.80, H; 11.08, N.

1-[5-(4-Nitrophenyl)-3-(4-fluorophenyl)-1,3-thiazol-2(3H)-ylidene]-3-[3-fluoro-4-(morpholin-4-yl)phenyl]thiourea (6): A mixture of compound 4 (10 mmol) and 4-nitrophenacylbromide (10 mmol) in absolute ethanol was refluxed in the presence of dried sodium acetate (50 mmol) for 8 h. The reaction content was then cooled to room temperature and the salt was separated by filtration. After the solvent was removed under reduced pressure, a solid appeared. This crude product was recrystallized from dimethyl sulfoxide to afford the desired product (yield: 2.44 g, 63%). Mp 206 °C; IR (KBr) cm⁻¹: 1725 (C=O), 1449 (C=N); 1H-NMR (DMSO-d₆): δ ppm 2.99-2.95 (brs, 4H, 2CH₂), 3.72 (s, 4H, 2CH₂), 4.12 (s, 2H, thiazole C₄), 6.68-6.74 (m, 3H, arH), 6.98-7.29 (m, 4H, arH); MS m/z (%): 117.87 (76), 117.99 (86), 148.71 (58), 148.84 (79), 152.91 (73), 153.09 (58), 154.72 (43), 155.10 (32), 214.79 (38), 214.91 (48), 229.05 (100), 229.24 (72), 230.87 (55), 270.16 (37), 271.16 (50), 273.10 (58), 284.18 (56), 300.20 (35), 370.27 (33), 371.34 (75), 383.29 (78), 384.36 (46), 413.39 ([M+1+Na]+, 60); Anal. Calcd. (%) for C₁₉H₁₇F₂N₃O₂S: 58.60, C; 4.40, H; 10.79, N. Found: 58.77, C; 4.80, H; 11.08, N.
δ ppm 2.94 (brs, 4H, 2CH₂), 3.72 (brs, 4H, 2CH₂), 5.48 (brs, 1H, thiazole C4), 6.69-7.14 (m, 6H, arH), 7.15-7.60 (m, 1H, arH), 7.69 (s, 1H, arH), 7.82-7.86 (m, 1H, arH), 8.13-8.17 (m, 2H, arH): ^1^C-NMR (DMSO-d₆): δ ppm 50.88-51.50 (2CH₂), 66.78-66.94 (2CH₂), arC [110.19 (CH), 117.13 (CH), 118.25 (CH), 120.58 (2CH), 123.94 (CH), 125.79 (CH), 129.07 (2CH), 129.85 (2CH), 131.48 (C), 132.25 (C), 137.73 (C), 137.92 (C), 147.57 (C), 147.80 (C), 157.19 (C)], 120.58 (thiadiazole C4), 138.49 (thiazole C5), 149.63 (thiazole C2); MS m/z (20): 195.88 (71), 208.90 (95), 309.15 (75), 331.18 (100); Anal. Calcd. (%) for C₁₁H₁₂N₂O₂S: 56.17, C; 7.18, H; 20.87, N. Found: 56.17, C; 7.19, H; 20.86, N.

**Ethyl [[3-fluoro-4-(morpholin-4-yl)phenyl]amino]acetate (7):** Ethyl bromoacetate (10 mmol) was added to a mixture of compound 2 (10 mmol) and triethylamine (10 mmol) dropwise in dry tetrahydrofuran at 0-5 °C. The reaction content was allowed to reach room temperature and was stirred for 11 h; the progress of the reaction was monitored by TLC. The precipitated triethylammonium salt was removed by filtration. After the solvent was evaporated under reduced pressure, a yellow solid appeared. This crude product was recrystallized from ethyl acetate and petroleum ether (1:2) to afford the desired product (yield: 1.94 g, 69%). Mp 85 °C; IR (KBr) cm⁻¹: 3384 (NH), 1729 (C=O), 1117 (C-O); ^1^H-NMR (DMSO-d₆): δ ppm 1.16 (t, 3H, CH₃, J = 6.6 Hz), 2.79 (brs, 4H, 2CH₂), 3.66 (brs, 4H, 2CH₂), 3.82 (s, 2H, NCH₂), 4.07 (q, 2H, OCH₂, J = 6.6 Hz), 5.93 (brs, 1H, NH, D₂O exchange), 6.25-6.40 (m, 2H, arH), 6.81 (t, 1H, arH, J = 9.0 Hz); ^1^C-NMR (DMSO-d₆): δ ppm 14.68 (CH), 34.69 (2CH₂), 51.37 (2CH₂), 101.01 (CH), 108.56 (CH), 121.01 (CH), 130.04 (C), 145.54 (C), 154.49 (C), 172.10 (C=O); MS m/z (%): 124.80 (43), 130.80 (100), 152.83 (64), 162.84 (42), 190.94 (37), 195.88 (68), 196.89 (54), 208.90 (38), 210.90 (23), 283.05 ([M+1]^+), 19, 383.24 (48); Anal. Calcd. (%) for C₅₂H₅₁F₄N₂O₅S: 60.72, C; 4.08, H; 11.33, N. Found: 60.33, C; 4.48, H; 11.30, N.

**2-[[3-Fluoro-4-(morpholin-4-yl)phenyl]amino]acetohydrazide (8):** Hydrazide hydrazide (25 mmol) was added to a solution of compound 7 (10 mmol) in absolute ethanol, and the mixture was allowed to reflux for 7 h. When the reaction mixture cooled to room temperature, a white solid appeared. The crude product was filtered off and recrystallized from ethanol to give the desired compound (yield: 1.87 g, 70%). Mp 182 °C; IR (KBr) cm⁻¹: 3339-3293 (NH), 1653 (C=O), 1226 (C=S). ^1^H-NMR (DMSO-d₆): δ ppm 2.93 (brs, 4H, 2CH₂), 3.70-3.73 (m, 4H, 2CH₂), 3.76 (d, 2H, CH₂, J = 9.8 Hz), 7.16 (t, 2H, arH, J = 5.2 Hz), 5.88 (s, 1H, NH, D₂O exchange), 6.34-6.48 (m, 2H, arH), 6.82 (t, 1H, arH, J = 9.8 Hz), 7.16 (t, 2H, arH, J = 9.0 Hz).

**N-(4-Fluorophenyl)-2-[[3-fluoro-4-(morpholin-4-yl)phenyl]amino]acyetyl hydrazinecarbothioamide (9):** A mixture of compound 8 (10 mmol) and 4-fluorophenyl isothiocyanate (10 mmol) in absolute ethanol was refluxed for 7 h. When the reaction content cooled to room temperature, a white solid formed. This crude product was filtered off and recrystallized from ethyl acetate to afford the desired compound (yield: 3.02 g, 61%). Mp 160 °C; IR (KBr) cm⁻¹: 3331 (2NH), 3164 (2NH), 1690 (C=O), 1226 (C=S). ^1^H-NMR (DMSO-d₆): δ ppm 2.79 (brs, 4H, 2CH₂), 3.41 (brs, 4H, 2CH₂), 3.76 (d, 2H, CH₂, J = 5.2 Hz), 5.88 (s, 1H, NH, D₂O exchange), 6.34-6.48 (m, 2H, arH), 6.82 (t, 1H, arH, J = 9.8 Hz), 7.16 (t, 2H, arH, J = 9.0 Hz).
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Hz), 7.37 (brs, 2H, arH), 9.57 (brs, 1H, NH, D$_2$O exchange), 9.69 (brs, 1H, NH, D$_2$O exchange), 10.10 (brs, 1H, NH, D$_2$O exchange); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ ppm 45.38 (CH$_2$), 51.61 (2CH$_2$), 66.40 (2CH$_2$), arC (100.57 (CH), 114.67 (2CH), 115.48 (2CH), 120.40 (CH), 135.33 (C), 145.11 (C), 153.97-157.24 (C), 158.40-158.79 (C), 160.04-162.06 (C)), 170.20 (C=O), 181.27 (C=S); MS (ESI) m/z (%): 104.73 (26), 122.73 (24), 137.77 (46), 150.75 (34), 164.80 (23), 190.81 (36), 195.83 (45), 208.87 (100), 268.94 (32), 403.11 (25), 421.10 ([M$^+$], 32), 422.17 ([M$^+$]+, 82), 423.17 ([M+Na$^+$]+, 20), 460.10 ([M+K$^+$]+, 61); Anal. Calcd. (%): for C$_{19}$H$_{21}$F$_2$N$_5$O$_2$S: 54.15, C; 5.02, H; 17.62, N; Found: 54.55, C; 5.22, H; 17.13, N.

4-(4-Fluorophenyl)-5-[[3-fluoro-4-(morpholin-4-yl)phenyl]amino]methyl)-4H-1,2,4-triazole-3-thiol (10): A solution of compound 9 (10 mmol) in ethanol and water (1:1) was refluxed in the presence of 2 N NaOH for 3 h, and then the resulting solution was cooled to room temperature and acidified to pH 4 with 37% HCl. The precipitate that formed was filtered off, washed with water, and recrystallized from ethyl acetate to afford the desired compound (yield: 2.47 g, 61%). Mp 211-213.9°C; IR (KBr) cm$^{-1}$: 3412 (NH), 3065 (aromatic CH), 1747 and 1709 (2C=O), 1450 (C=N); $^1$H-NMR (DMSO-$_d_6$): $\delta$ ppm 2.74 (s, 4H, 2CH$_2$), 3.67 (s, 4H, 2CH$_2$), 4.06 (s, 2H, CH$_2$), 5.82 (brs, 1H, NH, D$_2$O exchange), 6.17-6.31 (m, 2H, arH), 6.72 (t, 1H, arH, J = 8.8 Hz), 7.24-7.42 (m, 4H, arH), 13.80 (s, 1H, SH, D$_2$O exchange); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ ppm 38.82-41.35 (DMSO-$d_6$+CH$_2$), 52.08 (2CH$_2$), 67.00 (2CH$_2$), arC (101.02 (CH), 108.56 (CH), 116.7 (CH), 117.18 (CH), 120.88 (CH), 120.97 (CH), 131.04 (CH), 130.40 (C), 145.13 (C), 151.00 (C), 159.18-160.41 (C), 163.51-168.95 (C), 144.92 (triazole C3), 154.36 (triazole C5); MS m/z (%): 237.00 (21), 421.10 (39), 423.17 (M+1$^+$]+, 20), 460.10 ([M+K$^+$]+, 61); Anal. Calcd. (%): for C$_{19}$H$_{21}$F$_2$N$_5$O$_2$S: 54.15, C; 5.02, H; 17.62, N; Found: 54.55, C; 5.22, H; 17.13, N.

5-[[3-Fluoro-4-(morpholin-4-yl)phenyl]amino]methyl)-N-(4-fluorophenyl)-1,3,4-thiadiazole-2-amine (11): Concentrated sulfuric acid (64 mmol) was added to compound 9 (10 mmol) dropwise while stirring, and the reaction content was stirred in an ice bath for 15 min. The mixture was allowed to reach room temperature and was stirred for an additional 3 h. The resulting solution was then poured into ice-cold water and made alkaline (pH 8) with ammonia. The precipitated product was filtered, washed with water, and recrystallized from ethanol to afford the desired product (yield: 3.83 g, 95%). Mp 177 °C; IR (KBr) cm$^{-1}$: 3412 (NH), 3065 (arCH), 1227 (2CH), 1377 (2CH), 1507 (34), 1648 (23), 1908 (36), 1958 (45), 2088 (70), 2689 (32), 40311 (25), 42110 ([M$^+$]+, 32), 42217 ([M+Na$^+$]+, 82), 42317 ([M+K$^+$]+, 20), 46010 ([M+K$^+$]+, 61); Anal. Calcd. (%): for C$_{19}$H$_{19}$F$_2$N$_5$O$_5$: 56.56, C; 4.75, H; 17.36, N; Found: 56.96, C; 4.68, H; 17.13, N.

N'-[[2(Z)-3-(4-Fluorophenyl)-4-oxo-1,3-thiazolidin-2-ylidene]-2-[[3-fluoro-4-(morpholin-4-yl)phenyl]amino]acetohydrazide (12): Ethyl bromoacetate was added to a solution of compound 9 in absolute ethanol (10 mmol) and the mixture was refluxed for the dried sodium acetate (16.4 g, 200 mmol) for 9 h. The mixture was then cooled to room temperature, poured into ice-cold water while stirring, and left overnight in the cold. The formed solid was filtered, washed with water 3 times, and recrystallized from benzene and petroleum ether (1:2) to afford the pure desired compound (yield: 2.42 g, 52.5%). Mp 152 °C; IR (KBr) cm$^{-1}$: 3361 (NH), 3065 (aromatic CH), 1747 and 1709 (2C=O), 1450 (C=N); $^1$H-NMR (DMSO-$d_6$): $\delta$ ppm 2.74 (s, 4H, 2CH$_2$), 3.67 (s, 4H, 2CH$_2$), 4.06 (s, 2H, CH$_2$), 5.82 (brs, 1H, NH, D$_2$O exchange), 6.17-6.31 (m, 2H, arH), 6.72 (t, 1H, arH, J = 8.8 Hz), 7.24-7.42 (m, 4H, arH), 13.80 (s, 1H, SH, D$_2$O exchange); $^{13}$C-NMR (DMSO-$d_6$): $\delta$ ppm 38.82-41.35 (DMSO-$d_6$+CH$_2$), 52.08 (2CH$_2$), 67.00 (2CH$_2$), arC (101.02 (CH), 108.56 (CH), 116.7 (CH), 117.18 (CH), 120.88 (CH), 120.97 (CH), 131.04 (CH), 130.40 (C), 145.13 (C), 151.00 (C), 159.18-160.41 (C), 163.51-168.95 (C), 144.92 (triazole C3), 154.36 (triazole C5); MS m/z (%): 237.00 (31), 404.19 ([M+1$^+$]+, 100), 405.20 (29); Anal. Calcd. (%): for C$_{19}$H$_{19}$F$_2$N$_5$O$_5$: 56.56, C; 4.75, H; 17.36, N; Found: 56.66, C; 4.45, H; 17.16, N.
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2.80 (s, 4H, 2CH₂), 3.68 (s, 4H, 2CH₂), 4.14 (s, 2H, thiazole C4), 4.37 (s, 2H, CH₂), 6.12 (brs, 1H, NH, D₂O exchange), 6.39-6.47 (m, 2H, arH), 6.78-6.91 (brs, 2H, arH), 7.18-7.34 (brs, 3H, arH), 10.82 (brs, 1H, NH, D₂O exchange); ¹³C-NMR (DMSO-d₆): δ ppm 29.90 (thiazole C4), 51.41 (2CH₂), 45.45 (NCH₂), 66.28 (2CH₂), arC [101.05 (CH), 108.29 (CH), 116.18 (CH), 120.12 (CH), 120.20 (CH), 122.24 (CH), 122.40 (CH), 143.62 (C), 143.67 (C), 145.10 (C), 152.28 (C), 156.68 (C)], 153.75 (thiazole C2), 168.33 (exocyclic C=O), 169.22 (thiazole C5); MS m/z (%): 100.79 (84), 102.73 (72), 103.10 (68), 110.86 (31), 196.96 (43), 208.91 (43), 229.00 (44), 368.16 (45), 388.25 (20), 462.27 ([M+1]+, 100), 463.21 (25), 484.17 ([M+Na]+, 38), 500.13 ([M+K]+, 13); Anal. Calcd. (%) for C₂₁H₂₁F₂N₅O₃S: 54.66, C; 4.59, H; 15.18, N. Found: 54.26, C; 4.25, H; 15.58, N.

N’-[(2Z)-5-(4-Chlorophenyl)-3-(4-fluorophenyl)-1,3-thiazol-2(3H)-ylidene]-2-{{3-fluoro-4-(morpholin-4-yl)phenyl}amino}acetohydrazide (13): 4-Chlorophenacylbromide (10 mmol) and dried sodium acetate (16.4 g, 200 mmol) were added to a solution of compound 9 in absolute ethanol, and the reaction mixture was refluxed for 11 h. The mixture was cooled to room temperature, poured into ice-cold water while stirring, and left overnight in the cold. The formed solid was filtered, washed with water 3 times, and recrystallized from ethanol to afford the desired compound (yield: 1.37 g, 25.3%). Mp 156 °C; IR (KBr) cm⁻¹: 3373-3290 (2NH), 3076 (aromatic CH), 1698 (C=O); ¹H-NMR (DMSO-d₆): δ ppm 2.80 (s, 4H, 2CH₂), 3.67 (s, 6H, 3CH₂), 4.18 (brs, 1H, NH, D₂O exchange), 4.84 (brs, 1H, NH, D₂O exchange), 5.86 (brs, 1H, thiazole C4), 6.29-6.36 (m, 2H, arH ), 6.75 (brs, 1H, arH), 7.39-7.63 (m, 6H, arH), 7.97-8.01 (m, 2H, arH); ¹³C-NMR (DMSO-d₆): δ ppm 38.77-41.28 (DMSO-d₆+CH₂), 52.14 (2CH₂), 67.01 (2CH₂), arC [100.95 (CH), 101.44 (CH), 108.63 (CH), 116.75 (CH), 117.26 (CH), 120.93 (CH), 129.62 (CH), 130.13 (CH), 130.31 (CH), 134.56 (C), 139.38 (C), 145.19 (C), 145.40 (C), 151.08 (C), 154.37 (C), 154.65 (C), 159.19 (thiazole C5), 160.70 (thiazole C2), 165.62 (C=O); MS m/z (%): 555.33 (27), 556.27 ([M]+, 100), 557.22 ([M+1]+, 43), 563.57 (24), 578.24 ([M+1+Na]+, 84), 582.26 (69), 584.15 (26), 594.28 ([M+1+K]+, 76), 596.23 ([M+1+K]+, 42); Anal. Calcd. (%) for C₂₇H₂₄ClF₂N₅O₂S: 58.32, C; 4.35, H; 12.60, N. Found: 58.72, C; 4.75, H; 12.20, N.

Antimicrobial activity assessment

All test microorganisms were obtained from the Refik Saydam Hıfızıssıhha Institute (Ankara, Turkey) and were as follows: E. coli ATCC35218, E. aerogenes ATCC13048, Y. pseudotuberculosis ATCC911, P. aeruginosa ATCC43288, S. aureus ATCC25923, E. faecalis ATCC29212, B. cereus 709 Roma, M. smegmatis ATCC607, C. albicans ATCC60193, C. tropicalis ATCC 13803, A. niger RSKK 4017, and S. cerevisiae RSKK 251. All of the newly synthesized compounds were weighed and dissolved in dimethyl sulfoxide to prepare extract stock solutions of 5.000 mg mL⁻¹. A screening test using the agar-well diffusion method, as adapted earlier, was used for all newly synthesized compounds. Each microorganism was suspended in Mueller-Hinton (MH) broth (Difco, Detroit, MI, USA) and the solutions were diluted to approximately 106 cfu mL⁻¹. They were flood-inoculated onto the surface of MH agar and Sabouraud dextrose agar (SDA; Difco) and then dried. For C. albicans and C. tropicalis, SDA was used. Wells 5 mL in diameter were cut from the agar using a sterile cork borer, and 50 mL of the extracted substances was delivered into the wells. The plates were incubated for 18 h at 35 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (10 mg), streptomycin (10 mg), and fluconazole (5 mg) were the standard drugs. Dimethyl sulfoxide and ethanol were used as solvent controls. The antimicrobial activity results are summarized in Table 1.
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Table 1. Screening for antimicrobial activity of new compounds (mm).

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>Microorganisms and inhibition zones (mm)</th>
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<tr>
<td></td>
<td>Ec Ea Yp Pa Sa Ef Bc Ms Ca Sc</td>
</tr>
<tr>
<td>3</td>
<td>- - - 15 8 8 8 8 -</td>
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<tr>
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<tr>
<td>12</td>
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<td>13</td>
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<tr>
<td>Amp.</td>
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<tr>
<td>Strep.</td>
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<tr>
<td>Flu.</td>
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</table>


Results and discussion

The main aim of the present study was to synthesize and investigate the antimicrobial activities of linezolid-like molecules. Synthesis of the intermediate and target compounds was performed according to the reactions outlined in Figures 1 and 2.

The IR and $^1$H-NMR spectra of compound 3, obtained from the condensation of 2 with indol-3-carbaldehyde, contained no signals corresponding to an amine group. Instead, new signals due to indole-3-yl moiety were recorded at the related chemical shift values. Moreover, the IR spectrum of compound 3 showed an absorption band at 1443 cm$^{-1}$, indicating the presence of a CH=N bond in the ring. In addition, a singlet corresponding to 1 proton characteristic of the N=CH group was observed at 9.11 ppm in the $^1$H-NMR spectrum of compound 3. This group was recorded at 107.01 ppm in the $^{13}$C-NMR spectrum.

It has been reported that compounds with imine bonds may exist as $E/Z$ geometrical isomers about the N=CH double bond. A literature survey revealed that the compounds containing imine bonds are present in higher percentages in DMSO-d$_6$ solution in the form of the geometrical $E$ isomer about the N=CH double bond. The $^1$H-NMR and $^{13}$C-NMR spectra (in DMSO-d$_6$) of compound 3 confirmed its existence as an $E$ geometrical...
isomer, which exhibited NMR data consistent with the literature findings for analogous compounds containing the imine functionality.\textsuperscript{31–36}

The synthesis of 1-[3-fluoro-4-(morpholin-4-yl)phenyl]-3-(4-fluorophenyl)thiourea (4) was performed from the reaction of compound 2 with 4-fluorophenyl isothiocyanate, and the structure was confirmed based on spectroscopic methods. Thiourea derivative 4 was then converted to (Z)-2-(3-fluoro-4-morpholinophenylimino)-3-(4-fluorophenyl)thiazolidin-4-one (5) by cyclocondensation with ethyl bromoacetate, while the condensation
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Figure 2. Synthetic of compounds 7-13.
of the same intermediate (4) with 4-nitrophenacylbromide afforded (Z)-3-fluoro-N-(3-(4-fluorophenyl)-5-(4-nitrophenyl)thiazol-2(3H)-ylidene)-4-morpholinobenzenamine (6). In the $^1$H-NMR spectra of compounds 5 and 6, no signal pointing toward thiourea moiety was observed. Instead, a new signal due to the C4 proton (for 6) or C5 protons (for 5) of the 1,3-thiazole scaffold was recorded at 4.12 ppm (for 5) and 5.48 ppm (for 6), integrating for 2 protons and 1 proton, respectively. A satisfactory $^{13}$C-NMR spectrum for compound 5 was not obtained due to the slight solubility of 5 in all NMR solvents.

The treatment of compound 2 with ethyl bromoacetate in basic media produced ethyl {[3-fluoro-4-(morpholin-4-yl)phenyl]amino}acetate (7). Ester 7 was then converted to the corresponding hydrazide derivative, 2-{[3-fluoro-4-(morpholin-4-yl)phenyl]amino}acetohydrazide (8), via the substitution of the ethoxy group by hydrazine hydrate. Compound 7 was characterized by the presence of additional signals derived from the ester group, which were observed in the $^1$H-NMR spectrum of compound 7 at 1.16 ppm (-OCH$_2$CH$_3$) and 3.82 (-OCH$_2$CH$_3$) ppm, integrating for 3 and 2 protons, respectively. This group appeared at 14.68 and 61.18 ppm in the $^{13}$C-NMR spectrum. When compound 7 was converted to hydrazide 8, the signals that originated from ester functionality disappeared; instead, new signals due to the hydrazide structure were recorded at 4.25 and 9.11 ppm, integrating 2 protons and 1 proton, respectively, and controlled by D$_2$O exchange. Further support for the formation of the hydrazide structure is the appearance of strong absorption vibrations pointing toward NHNH$_2$ at 3339 and 3293 cm$^{-1}$ in the FTIR spectrum.

The treatment of 8 with 4-fluorophenyl isothiocyanate afforded corresponding carbothioamide derivative 9 under reflux conditions. In contrast to those of compound 8, the $^1$H- and $^{13}$C-NMR spectra of compound 9 exhibited additional signals due to carbothioamide moiety at the related chemical shift values. In addition, compound 9 had relatively stable [M$^+$] and [M+1]$^+$ ion peaks in its mass spectrum.

The treatment of compound 9 with 2 N NaOH caused the conversion of the carbothioamide side chain into a 1,2,4-triazole ring; thus, 5-{[3-fluoro-4-(morpholin-4-yl)phenyl]amino}methyl)-4-(4-fluorophenyl)-H-1,2,4-triazole-3-thiol (10) was obtained. The cyclization of the same intermediate (9) in the presence of cold concentrated sulfuric acid produced 5-(3-fluoro-4-morpholinophenlamino)methyl)-N-(4-fluorophenyl)-1,3,4-thiadiazol-2-amine (11). In the FTIR spectrum of compound 10, the –NH- stretching band appeared at 3412 cm$^{-1}$. The –NH- proton resonated at 5.82 ppm in the $^1$H-NMR spectrum of compound 10 as a broad singlet. Moreover, the IR spectrum of compound 10 displayed a -SH stretching band at 2850 cm$^{-1}$. This group resonated at 13.80 ppm in the $^1$H-NMR spectrum of compound 10, controlled by D$_2$O exchange. In the FTIR and $^1$H-NMR spectra of compound 11, no signal derived from the –SH function was recorded, while 2 signals pointing toward the presence of 2 –NH- groups were observed in the related frequencies and the chemical shift values were consistent with the literature. Furthermore, the mass spectra of these compounds exhibited stable [M+1]$^+$ ion peaks.

The synthesis of compounds 12 and 13 was carried out by the reaction of compound 9 with 4-chlorophenacylbromide (for 13) or ethyl bromoacetate (for 12) in ethanolic solution in the presence of dried sodium acetate under reflux conditions.

The FTIR spectra of compounds 12 and 13 displayed a signal at 1698 (for 13) and 1702 (for 12) cm$^{-1}$ pointing toward the presence of the carbonyl function. Moreover, in the $^1$H-NMR spectra of compounds 12 and 13, no signal derived from the -SH group was observed; instead, new signals due to 2 –NH- groups were detected.
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at 6.12 or 10.82 ppm (for 12) and 4.18 ppm or 9.37 ppm (for 13), exchangeable with D₂O. Furthermore, the C-4 proton(s) of the 1,3-thiazole scaffold appeared at 4.14 (for 12) and 5.86 ppm (for 13), integrating 2 protons and 1 proton, respectively.

All of the newly synthesized compounds had elemental analysis results consistent with the assigned structures.

Additional support for the formation of the targeted compounds was obtained from the appearance of [M]⁺ or [M+1]⁺ ion peaks at corresponding m/z values, confirming their molecular masses.

Hantzsch synthesis, involving the reaction of α-halocarbonyl compounds with thioamides, is the most commonly applied method,37,38 although several other methods have been developed for the preparation of 1,3-thiazoles. The Hantzsch reaction starts with the attack of the sulfur atom of thioamide, which is present in its enethiol form, on the halogen-containing atom of phenacyl bromide, and then the elimination of HBr and H₂O leads to the 1,3-thiazole ring.38

Because compound 9 possess more than 1 nucleophilic center and phenacyl bromide has 2 positions for nucleophilic attacks, there are at least 4 different possibilities leading to the formation of 4 different structural isomers; each of them can exist as their individual E and Z geometrical isomers (Figure 3).

To identify the exact structure of compound 13, full geometric optimization of possible products 13-16 was obtained by the DFT/B3LYP39,40 method with the 6-31G(d,p) basis set, and the structure of the molecules was also investigated in detail. The solvent effect was evaluated using the conducting polarized continuum model (CPCM).41,42 Using the optimized geometries of the molecules at the B3LYP/6-31G(d,p) level, their single-point energies were computed using the CPCM-B3LYP/6-31G(d,p) method. The calculated relative energies are given in Table 2.

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>Relative energy (kcal mol⁻¹)</th>
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<tr>
<td>E-15</td>
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</table>

According to the obtained results, the most stable product is the Z geometrical isomer of structure 13, with a calculated relative energy of 0.000 kcal mol⁻¹. Therefore, thermodynamically, the formation of the Z isomers of 13 is more favorable. Although a type of 16 isomers has been reported in the literature as reaction products between thioamides and phenacyl bromides,43,44 the calculated relative energy for the Z geometrical isomer of 16, in the range of 4.243 to 5.543 kcal mol⁻¹, indicates that this isomer is less favorable than 13. Although the stability of the Z geometrical isomer of 14, with the calculated relative energy of 0.339 and 0.328 kcal mol⁻¹, is close to the Z geometrical isomer of 13, the existence of only 1 spot in the TLC plate of compound 13 strongly supports the formation of the Z geometrical isomer of 13 as the unique reaction product.
Figure 3. Possible structural and geometrical isomers for compounds 13.
Figure 4. Mechanism leading to the formation of compounds 6 and 13.
Moreover, the melting point and NMR spectral data point to the formation of only one product. Contrary to the description in the literature, the reaction between carbothioamide 9 and phenacyl bromide began with the attack of the sulfur atom on the carbonyl carbon of phenacyl bromide instead of the halogen-bearing carbon atom of the acyl component, because the latter attack leads to the formation of 16, which is a less favorable isomer due to its higher energy content (Figure 4).

It has been speculated that in 16 the bulky groups are positioned too close to each other, causing a less stable structure, as seen in Table 2. The Z rearrangement of the groups in compound 13 is likely due to the steric hindrance of the bulky R group and the fluorophenyl moiety in isothiocyanate intermediate A. Based on the same idea, it can be concluded that compounds 5, 6, and 12 exist as their individual Z isomers. In the reaction media, the presence of dried sodium acetate is necessary to accelerate the reaction by catching the \( \text{H}_2\text{O} \) and \( \text{HBr} \) furnished during the reaction. In the same manner, compound 6 can be considered to exist as its Z isomer. Similarly, due to the hindrance between the fluorophenyl ring and other bulky groups incorporating morpholin-4-ylphenyl moiety, compounds 5 and 12 exist as their Z geometrical isomers.

All of the newly synthesized compounds were tested for their antimicrobial activities and the obtained results are presented in Table 1. The substitution of the 1,3-oxazol-2-one scaffold in the structure of linezolid by the 1,3-thiazole nucleus caused the loss of antimicrobial activity against gram-positive bacteria, whereas linezolid has been used as an antibiotic for infectious diseases caused by gram-positive pathogens. As seen in Table 1, only moderate activity against \( \text{Pseudomonas aeruginosa} \) (Pa), a gram-negative bacillus, was detected for compound 6 with an inhibition zone of 10 mm. In addition, slight activities against yeast like \( \text{Candida albicans} \) (Ca) and \( \text{Saccharomyces cerevisiae} \) (Sc) were observed for compounds 3, 5, and 12, with inhibition zones between 6 and 10 mm. Moreover, compound 3 displayed moderate activities against \( \text{Staphylococcus aureus} \) (Sa) and \( \text{Enterococcus faecalis} \) (Ef), which are gram-positive cocci, and \( \text{Bacillus cereus} \) (Bc), a gram-positive spore bacillus, with inhibition zones of 15, 8, and 8 mm, respectively. On the other hand, this structural modification of the linezolid molecule resulted in the emergence of activity against \( \text{Mycobacterium smegmatis} \) (Ms), a nonpigmented, rapidly growing mycobacterium and an atypical tuberculosis factor leading to morbidity and mortality. The highest activities, with inhibition zones of 20 mm, were observed for compounds 11 and 13, which contain a 4-oxo-1,3-thiazolidine nucleus or 5-(4-fluorophenyl)-1,3,4-thiadiazole, respectively, in the core structure of linezolid.

Acknowledgment

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