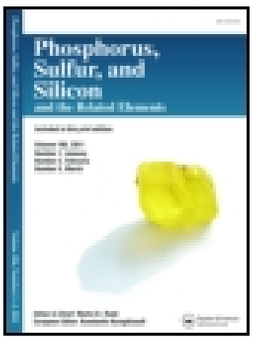

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Synthesis, characterization and biological activities evaluation of novel sulfanyl derivatives

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ABSTRACT

A novel series of 1-(3-Methyl-3-mesityl)-cyclobutyl-2-{[5-(2-fluorophenyl)-4-(aryl-alkyl)-4H-[1,2,4]triazol-3-yl]sulfanyl}-ethanone compounds were synthesized by a condensation reaction. The new compounds were characterized by elemental analyses, FT-IR and ^{1}H, ^{13}C NMR techniques. The antioxidant and antibacterial properties of the synthesized compounds were also investigated. The in vitro antioxidant and antibacterial activities of the newly synthesized compounds were measured, and they were found to exhibit significantly high antioxidant activity.

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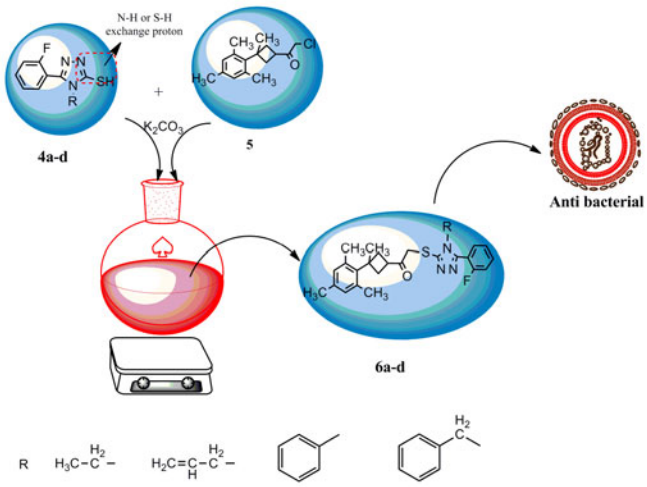
Sulfanyl compounds; 1,2,4-Triazole; IR and NMR spectroscopy; biological effects

GRAPHICAL ABSTRACT



Introduction

The ring-closure reaction of carbohydrazide is well known. The ring systems of these structures are typically planar 6π–electron aromatic systems and are used as starting materials for the synthesis of many heterocycles.[1–4] The chemistry of 1,2,4-triazoles has received considerable attention owing to their synthetic utility and wide spectrum of biological activities. Many studies have shown that 1,2,4-triazoles have potent biological characteristics such as antimicrobial,[5,6] antifungal,[7,8] antibacterial,[9–12] anticancer,[13] antimycobacterial, antiviral,[14,15] antimycotic activity,[16–18] antitubercular,[19] anticonvulsants,[20] antinociceptive,[21] antioxidant,[22–24] anti-inflammatory and analgesic [25] characteristics.

This study focuses on a detailed study of the synthesis, characterization, and antioxidant and antibacterial activities of 1-(3-Methyl-3-mesityl)-cyclobutyl-2-{[5-(2-fluorophenyl)-4-(aryl-alkyl)-4H-[1, 2, 4]triazol-3-yl]sulfanyl}-ethanone.

Results and discussion

Chemistry

The reaction sequences employed for the synthesis of the title compounds are shown in Figure 1.[26]

Reaction analysis of the title compounds (4a–d)

In the first step of this study, which was planned in two stages, four thiosemicarbazides (3a–d) were obtained in total by the interaction of 2-fluorobenzo hydrazide (1) with four different

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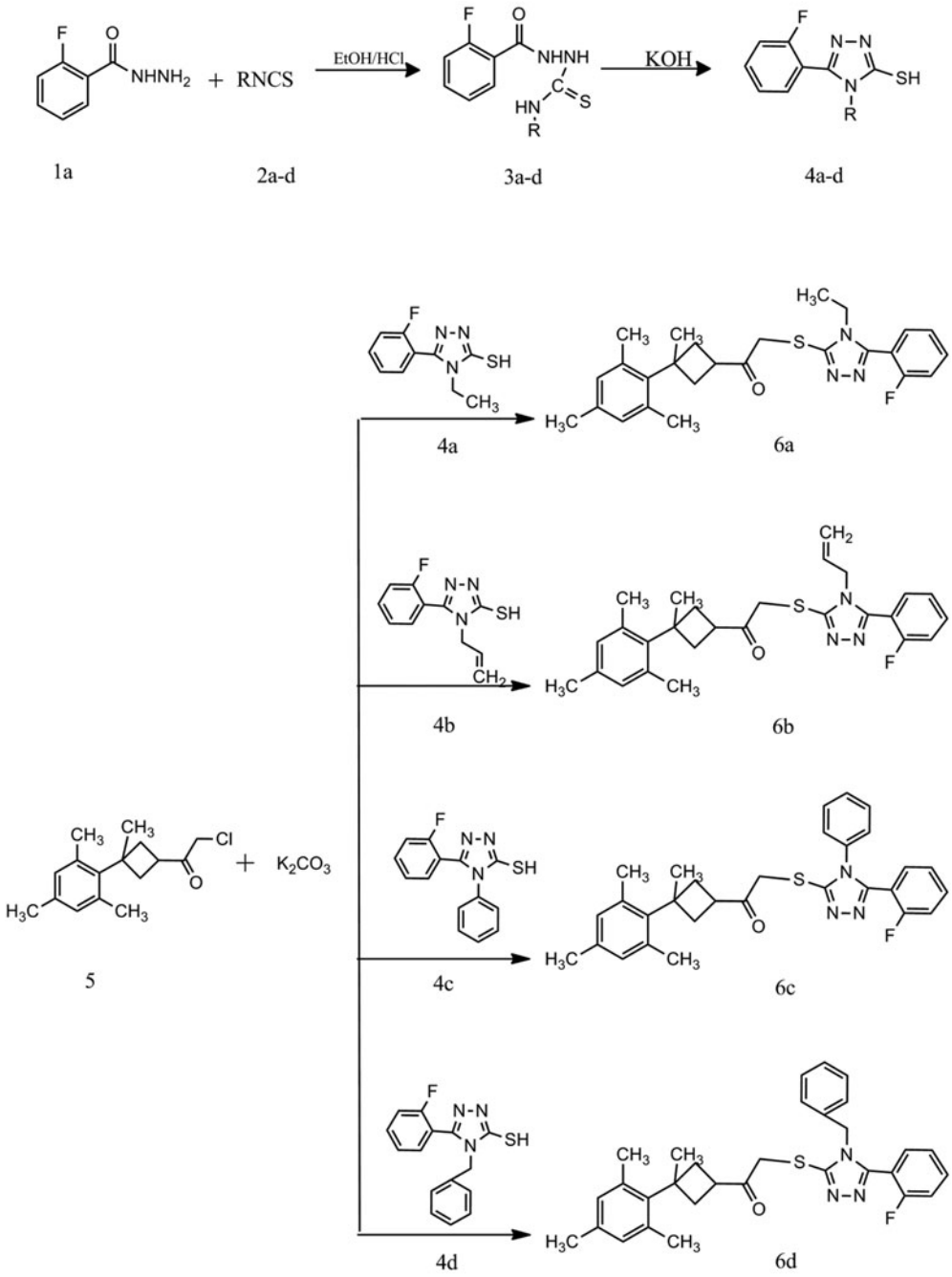

Figure 1. Synthesis and the structures of the compounds.

isothiocyanate derivatives (2a–d). KOH was subsequently added to the reaction medium to obtain 4-substituted-5-(2-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones (4a–d).[26] Carboxylic acid hydrazide derivatives form thiosemicarbazide derivatives by reacting to nucleophilic substitution with substituted isothiocyanates. The proposed reaction mechanisms of thiosemicarbazide derivatives are given in Figure 2.[27]

The nonbonding electrons in the nitrogen atom of the thiosemicarbazide in the basic medium cause the ring closure reaction by the thiosemicarbazide attacking the number one acyl carbonyl, and 4-substituted-1,2,4-triazole-3-thione derivatives (4a–d) are formed by the separation of one-mole water from the structure. The proposed mechanism of the formation of 4a–d is given in Figure 3.[28]

Reaction analysis of sulfanyl compounds containing triazole and cyclobutane ring (6a–d)

In the second step of the study, the compounds (4a–d) were reacted with 2-chloro-1-(3-methyl-3-mesitylcyclobutyl) (5) in dry acetone containing K_2CO_3 to give cyclobutane ring-, 4-substituted 1,2,4-triazole sulfanyl compounds (6a–d). The reaction mechanism of 6a–d is given in Figure 4.[29]

FT-IR analysis of synthesized compounds

When the FT-IR spectra of the synthesized 4-substituted 1,2,4-triazoles were examined, it was found that the C=O peak in the carboxylic acid hydrazides between 1650 and

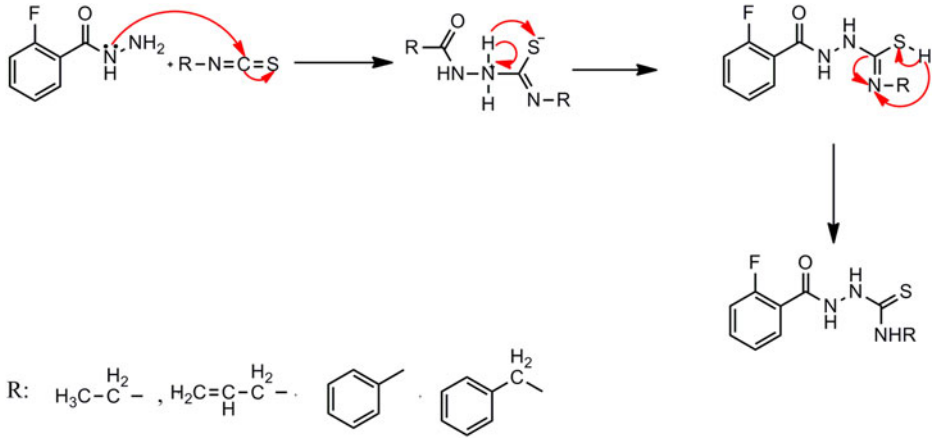

Figure 2. Reaction mechanism of thiosemicarbazide derivatives.

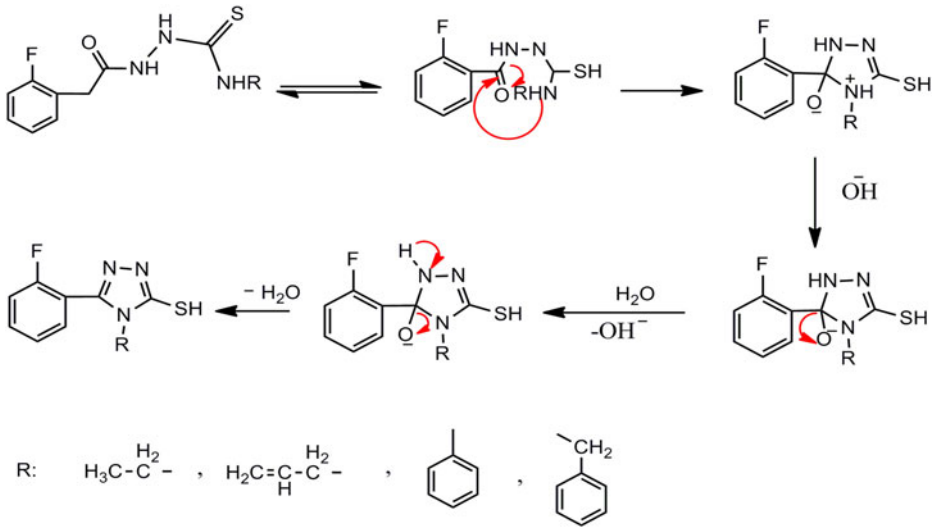

Figure 3. Mechanism of formation of 4a–d.

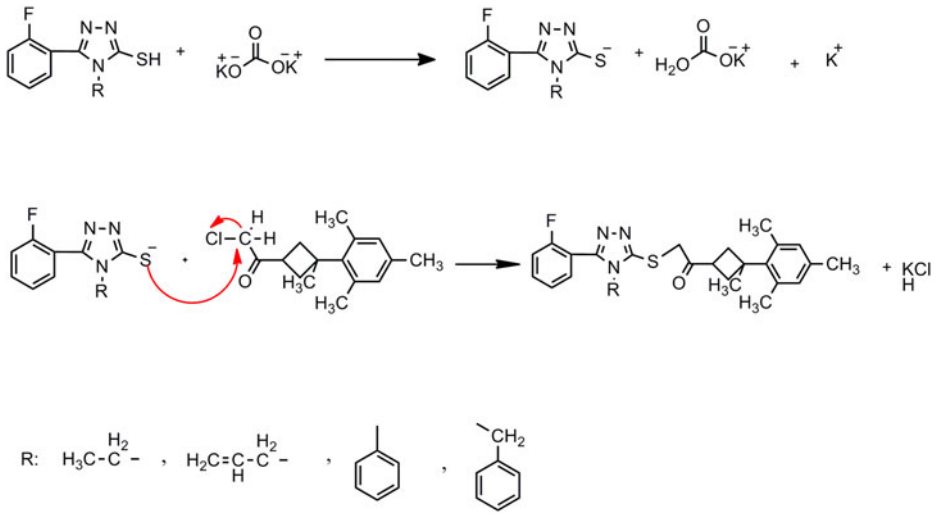

Figure 4. Mechanism of formation of 6a–d.

1690 cm^{-1} disappeared. Instead of this peak, N–C=S peaks at 1574, 1268, 1074, and 990 cm^{-1} appeared. The N–C=S vibration frequencies of 4-benzyl-5-(2-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione are shown in Figure S1 (Supplemental Materials). In addition to these characteristic peaks, aromatic C–H between 2987 and 3103 cm^{-1}, aliphatic C–H between 2852 and 2998 cm^{-1}, and C=N peaks around 1625 cm^{-1} appeared in 4-substituted 1,2,4-triazole derivatives.

In these synthesized compounds, the C=O stretching vibration appeared between 1705 and 1720 cm^{-1}. The

C–S–C stretching vibration of the structure was around 720 cm^{-1}. Some other important peaks were at Ar–H between 2950 and 3100 cm^{-1}, aliphatic CH between 2860 and 2920 cm^{-1}, C–C peaks at 1160 cm^{-1} at the cyclobutane ring, C=C peaks of the mesityl ring around 1450 cm^{-1}.

^{1}H and ^{13}C-NMR analysis of synthesized compounds

The most characteristic peak of the synthesized 4-substituted 1,2,4-triazoles was the SH/NH peak, which appeared as a singlet in the range of 14.10–14.24 ppm. The SH/NH peak for products 4a–d are given in Figure S2 (Supplemental Materials). In addition to this characteristic peak of SH/NH, there were some characteristic peaks in the substituents attached to the 1,2,4-triazole ring. The first example of these substituents was the ethyl fragment. The $–CH_2–$ protons of the $N–CH_2–CH_3$ in the 4-position were found to give a quartet peak at 3.86 ppm. The *J* value of this quartet peak was 7.1 Hz. The $–CH_3$ peaks showed triplet peak at 1.09 ppm. The *J* value of this triplet peak was 7.1 Hz. The carbons in the ^{13}C NMR spectrum of the ethyl group were at about 13.6 ppm for CH_3 and 39.6 ppm for CH_2. Because of the high electronegativity of the nitrogen atom, carbon, and hydrogen atoms close to the nitrogen atom appeared to be downfield. Thus, the electron charge density shifted from these atoms toward the nitrogen atom, and these atoms resonated in downfield.

The second example of these substituents is the allyl fragment. The peaks of the protons ($N–CH_2–$) adjacent to the $–N–CH_2–CH_2–CH_2$ nitrogen atom were observed at 4.52 ppm as a multiplet. (CH) proton of the $–CH_2–CH=CH_2$ was observed to peak between 5.64 and 5.70 ppm as a doublet of doublet of triplets (ddt). Trans proton of CH_2 in the $–CH_2–CH=CH_2$ appeared as a doublet at 4.73 ppm, and *cis* proton of CH_2 in the $–CH_2–CH=CH_2$ appeared as a doublet at 5.00 ppm.

The third example of these substituents is the phenyl fragment. The protons of the phenyl group appeared as a multiplet signal between 7.36 and 7.54 ppm. The final example of these substituents is the benzyl fragment. When ^{1}H-NMR of the benzyl group hydrogens is interpreted, aliphatic $N–CH_2–Ph$ hydrogens and aromatic hydrogens can be evaluated as two separate groups. The aliphatic protons appeared as singlets at 5.16 ppm downfield, which was considered to be low compared to other aliphatic protons, as they are both adjacent to the nitrogen atom high electronegativities and to the aromatic phenyl group. Protons in the meta positions from the aromatic hydrogens of the benzyl group appeared as multiple signals between 6.88 and 6.90 ppm, a proton in the para position appeared as a triplet at about 7.31 ppm, and hydrogens in the ortho positions appeared as triplets around 7.27 ppm.

Another characteristic peak in the ^{1}H-NMR spectrum of the 4-substituted 1,2,4-triazole sulfanyl compound was protons in the $–S–CH_2–CO$ fragment. These hydrogens appeared as a signal at about 4.32 ppm. The hydrogen that appeared as a pentet signal in the cyclobutane ring was one of the characteristic peaks in these structures. This proton, which interacts with four hydrogens in the cyclobutane ring, appeared as a signal of about 3.58 ppm. On the other hand, one of the characteristic peaks in the 4-substituted 1,2,4-triazole sulfanyl compounds was the signal that the nine $–CH_3$ protons in the mesitylene ring formed. This signal appeared as a singlet at about 2.14 ppm and was found to be equivalent to nine hydrogens. Another signal in the cyclobutane ring was the $–CH_3$ signal, which was a singlet at about 1.50 ppm. The Supplemental Materials contains sample ^{1}H-NMR spectra for the products (known compounds) 6a–d (Figures S14, S17, S20 and S23).

Biological

Biological activities of the synthesized compounds

After the synthesis of the compounds, we elucidated their biological activities with analytical and spectroscopic methods. Antibacterial activities were carried out according to the standard broth dilution method prescribed by the *Clinical and Laboratory Standards Institute* (CLSI).[30]

The Minimum Inhibition Concentration (MIC) values of the synthesized compounds were calculated against *B. subtilis* (ATCC 6633), *Listeria monocytogenes* Scott A strains as Gram-positive bacteria, *Proteus vulgaris* (FMC 1), *S. typhimurium* (NRRL B 4420) as Gram-negative bacteria, and these values are given in Table S1 (Supplemental Materials). It was observed that the antibacterial effects of the compounds varied between 64 and 1024 μg mL^{-1}. Among the compounds synthesized, the compound with the highest antibacterial activity (6b) was effective against *L. monocytogenes* and *P. vulgaris* strains at 64 μg mL^{-1}. Considering the substituents on the most active compounds against *L. monocytogenes* and *P. vulgaris*, the presence of the phenyl compound (6c) is particularly striking. Despite the very similar structures of all the compounds, different antibacterial effects suggest that they were due to the presence of different substituents. The antioxidant activity of the compound synthesized in the study was assessed using the free radical scavenging activity method. The reduction ability of DPPH radicals was determined by a decrease in absorbance at 517 nm induced by antioxidants and calculated percent inhibition values. The obtained findings are given in Table S2. It was determined that 6c showed better activity than the standard antioxidant BHT. On the other hand, it was found that it had a close activity relative to the α-tocopherol standard. According to this, it can be said that 6c has a good radical scavenging activity. This result can show that our test compound has good activity as a hydrogen donor because the antioxidants' effect on DPPH radical cleansing is thought to be due to their hydrogen donor capabilities.[31]

Experimental

Chemistry assays

Melting points were determined on Gallenkamp melting point apparatus. The IR spectra were measured with a Perkin–Elmer Spectrum one FT-IR spectrophotometer. The ^{1}H and ^{13}C-NMR spectra were recorded on Bruker AC-400 NMR spectrometer operating at 400 MHz for ^{1}H, 100 MHz

for ^{13}C-NMR. Compounds were dissolved DMSO, and chemical shifts were referenced to TMS (^{1}H and ^{13}C-NMR). Chemicals were purchased from Aldrich or Merck. The Supplemental Material contains FT-IR, ^{1}H-, and ^{13}C-NMR spectra for compounds 4a–d and 6a–d (Figures S1–S24).

General procedure for the synthesis of 4a–d

A mixture of 2-fluorobenzo hydrazide (1) (0.01 mol), ethyl alcohol (50 mL), and substituted isothiocyanate (2a–e) were refluxed for 3 h and after about 4 h, solid thiosemicarbazide began to form in the reaction flask. KOH (0.15 mol) was added to the solid, and dissolution was started. After 6 h, the reaction was stopped and brought to pH 3–4 with HCl. The residue was poured onto crushed ice while stirring. The resulting solid was collected by filtration, dried, and recrystallized from ethyl alcohol.

Synthesis of 4-ethyl-5-(2-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4a) ($C_{10}H_{10}FN_3S$)

White solid, yield 68%, m.p. 193–194 °C; FT-IR (KBr, cm^{-1}, υ): 3050–3100 (Ar–H), 2860–2984 (C–H), 1623 (C=N), 1227 (C–F), 1563, 1263, 1031, 964 (N–C=S, I, II, III ve IV amide bands); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ, ppm): 1.09 (t, 3H, N–CH$_2$–CH$_3$, J = 7.1 Hz), 3.86 (q, 2H, –N–CH$_2$–CH$_3$, J = 7.1 Hz), 7.43 (t, 1H, Ar–H, J = 7.5 Hz), 7.49 (t, 1H, Ar–H, J = 7.5 Hz), 7.70 (dt, 2H, Ar–H, J = 7.3 Hz, J = 19.7 Hz), 14.10 (s, 1H, SH); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ, ppm): 13.6, 39.6, 114.4, 116.7, 125.7, 132.5, 134.3, 146.7 (N–C–N), 161.4 (C–F), 167.2 (C=S). Elemental analysis: Calculated: C, 53.79; H, 4.51; N, 18.82; S, 14.36. Found: C, 53.70; H, 4.45; N, 18.85; S, 14.40.

Synthesis of 4-allyl-5-(2-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4 b) ($C_{11}H_{10}FN_3S$)

White solid, yield 65%, m.p. 202–204 °C; FT-IR (KBr, cm^{-1}, υ): 3053–3098 (Ar–H), 2852–2992 (C–H), 1630 (C=N), 1226 (C–F), 1562, 1264, 1035, 979 (N–C=S, I, II, III ve IV amide bands); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ, ppm): 4.52 (d, 2H, –N–CH$_2$–CH–CH$_2$, J = 5.0 Hz), 4.73 (d, 1H, –N–CH$_2$–CH–CH$_2$ (trans) J = 17.2 Hz), 5.00 (d, 1H, N–CH$_2$–CH–CH$_2$, J_{cis}=9.7 Hz), 5.64–5.70 (ddt, 1H, N–CH$_2$–CH–CH$_2$, J_{trans}=17.2 Hz, J_{cis}=9.7 Hz J CH2 = 5.0 Hz), 7.36 (t, 1H, Ar–H, J = 7.6 Hz), 7.41 (t, 1H, Ar–H, J = 9.3 Hz), 7.61 (t, 1H, Ar–H, J = 7.3 Hz), 7.65 (d, 1H, Ar–H, J = 13.3 Hz, J = 6.6 Hz) 14.11 (s, 1 H, SH); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ, ppm): 46.2, 114.4, 116.8, 117.9, 125.5, 131.5 132.2, 134.1, 146.9 (N–C–N), 160.9 (C–F), 167.8 (C=S). Elemental analysis: Calculated: C, 56.15; H, 4.28; N, 17.86; S, 13.63. Found: C, 56.23; H, 4.25; N, 17.82; S, 13.70.

Synthesis of 4-phenyl-5-(2-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4c) ($C_{14}H_{10}FN_3S$)

White solid, yield 72%, m.p. 111–112 °C; FT-IR (KBr, cm^{-1}, υ): 2987–3088 (Ar–H), 1623 (C=N), 1226 (C–F), 1548, 1278, 1076, 971 (N–C=S, I, II, III ve IV amide bands); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ, ppm): 7.18 (t, 1H, Ar–H, J = 9.2 Hz), 7.23 (t, 1H, Ar–H, J = 7.6 Hz), 7.27 (d, 2H, Ar–H, J = 7.4 Hz), 7.36–7.40 (m, 3H, Ar–H) 7.48–7.54 (m, 2H, Ar–H), 14.24 (s, 1H, SH); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ, ppm): 114.4, 116.2, 125.2, 128.3, 129.3, 129.6, 132.5, 134.8, 147.1 (N–C–N), 158.9 (C–F), 168.7 (C=S). Elemental analysis: Calculated: C, 61.98; H, 3.72; N, 15.49; S, 11.82. Found: C, 61.70; H, 3.60; N, 15.55; S, 11.90.

Synthesis of 4-benzyl-5-(2-fluorophenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (4d) ($C_{15}H_{12}FN_3S$)

White solid, yield 75%, m.p. 223–224 °C; FT-IR (KBr, cm^{-1}, υ): 3035–3103 (Ar–H), 2922–2998 (C–H), 1626 (C=N), 1241 (C–F) 1574, 1268, 1074, 990 (N–C=S, I, II, III ve IV amide bands); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ, ppm): 5.16 (s, 2H, N–CH$_2$–C$_6$H$_5$), 6.88–6.90 (m, 2H, Ar–H), 7.15–7.17 (m, 3H, Ar–H) 7.27 (t, 2H, Ar–H, J = 7.6 Hz), 7.31 (t, 1H, Ar–H, J = 9.2 Hz), 7.44 (t, 1H, Ar–H, J = 7.3 Hz), 7.59 (dd, 1H, Ar–H, J = 14.1 Hz, J = 7.0 Hz), 14.21 (s, 1H, SH); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ, ppm): 47.0, 114.4, 116.5, 125.4, 127.3, 128.0, 128.8, 132.0, 134.1, 135.7, 146.9 (N–C–N), 159.2 (C–F), 168.3 (C=S). Elemental analysis: Calculated: C, 63.14; H, 4.24; N, 14.73; S, 11.24. Found: C, 63.25; H, 4.20; N, 14.80; S, 11.40.

General procedure for the synthesis of 6a–d

K_2CO_3 (0.02 mol) was dissolved in dry acetone (30 mL). 2-chloro-1-(3-methyl-3-mesityl cyclobutyl) ethanone (0.02 mol) was added to this solution. A solution of appropriate triazoles (4a–d) (0.02 mol) was then added dropwise to this solution for 6 h at room temperature. The resulting solid was collected by filtration, dried, and recrystallized from ethyl alcohol.

Synthesis of 1-(3-methyl-3-mesityl)-cyclobutyl-2-{[5-(2-fluorophenyl)-4-ethyl-4H-1,2,4-triazol-3-yl] sulfanyl}-ethanone (6a) ($C_{26}H_{30}FN_3OS$)

Shining white solid, yield 62%, m.p. 150–152 °C; FT-IR (KBr, cm^{-1}, υ): 2980–3083 (Ar–H), 2861–2942 (C–H), 1720 (C=O), 1454 (C=C), 1372 (C=N), 1224 (C–F), 1024 (N–N), 720 (C–S); ^{1}H-NMR (400 MHz, DMSO-d$_6$, δ, ppm): 1.26 (t, 3H, N–CH$_2$–CH$_3$, J = 7.1 Hz), 1.50 (s, 3H, –CH$_3$ (cyclobutane)), 2.14 (s, 9H, –CH$_3$ (mesitylene)), 2.42–2.54 (m, 4H, –CH$_2$– (cyclobutane)), 3.58 (p, 1H, –CH– (cyclobutane), J = 8.80 Hz), 4.09 (q, 2H, N–CH$_2$–CH$_3$, J = 6.9 Hz), 4.35 (s, 2H, S–CH$_2$–), 6.70 (s, 2H, Ar–H (mesitylene)), 7.36–7.45 (m, 2H, Ar–H), 7.51–7.54 (m, 1H, Ar–H), 7.62–7.68 (m, 2H, Ar–H). ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ, ppm): 15.4, 20.5, 21.4, 25.2, 39.1, 40.3, 41.2, 115.4, 116.5, 127.1, 130.2, 130.3, 130.5, 132.5, 143.7, 150.9 (N–C–N), 151.8 (N–C–S), 160.8 (C–F), 205.3 (C=O). Elemental analysis: Calculated: C, 69.15; H, 6.70; N, 9.30; S, 7.10. Found: C, 69.01; H, 6.90; N, 9.42; S, 7.25.

Synthesis of 1-(3-methyl-3-mesityl)-cyclobutyl-2-{[5-(2-fluorophenyl)-4-allyl-4H-1,2,4-triazol-3-yl] sulfanyl}-ethanone (6b) ($C_{27}H_{30}FN_3OS$)

White solid, yield 64%, m.p. 91.3 °C; FT-IR (KBr, cm^{-1}, υ): 2975–3080 (Ar–H), 2855–2945 (C–H), 1715 (C=O), 1440

(C=C), 1380 (C=N), 1217 (C–F), 1012 (N–N), 732 (C–S); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 1.50 (s, 3H, –CH_3 (cyclobutane)), 2.14 (s, 9H, –CH_3 (mesitylene)), 2.46–2.54 (m, 4H, –CH_2– (cyclobutane)), 3.58 (p, 1H, –CH– (cyclobutane), J = 9.00 Hz), 4.31 (s, 2H, S–CH_2–), 4.44–4.52 (m, 2H, –N–CH_2–CH–CH_2), 4.81 (d, 1H, –N–CH_2–CH–CH_2(trans) J = 17.2 Hz), 5.25 (d, 1H, N–CH_2–CH–CH_2, J_{cis}=10.4 Hz), 5.74–5.83 (m, 1H, N–CH_2–CH–CH_2), 6.70 (s, 2H, Ar–H (mesitylene)), 7.36–7.45 (m, 2H, Ar–H), 7.53 (t, 1H, Ar–H, J = 7.3 Hz), 7.65 (dd, 2H, Ar–H, J_1=13.7 Hz, J_2=7.2 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 20.5, 21.4, 25.2, 39.1, 41.3, 46.8, 115.3, 116.6, 118.0, 125.6, 130.5, 132.1, 132.4, 133.5, 134.4, 134.9, 143.8, 150.5 (N–C–N), 151.1 (N–C–S), 161.1 (C–F), 205.3 (C=O). Elemental analysis: Calculated: C, 69.95; H, 6.52; N, 9.06; S, 6.92. Found: C, 69.81; H, 6.44; N, 9.15; S, 6.83.

Synthesis of 1-(3-methyl-3-mesityl)-cyclobutyl-2-{[5-(2-fluorophenyl)-4-phenyl-4H-1,2,4-triazol-3-yl] sulfanyl}-ethanone (6c) ($C_{30}H_{30}FN_3OS$)

White solid, yield 68%, m.p. 101–103 °C; FT-IR (KBr, cm^{-1}, υ): 2951–3059 (Ar–H), 2861–2920 (C–H), 1705 (C=O), 1426 (C=C), 1329 (C=N), 1224 (C–F), 1009 (N–N), 759 (C–S); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 1.50 (s, 3H, –CH_3 (cyclobutane)), 2.14 (s, 9H, –CH_3 (mesitylene)), 2.42–2.58 (m, 4H, –CH_2– (cyclobutane)), 3.58 (p, 1H, –CH– (cyclobutane), J = 8.90 Hz) , 4.30 (s, 2H, S–CH_2–), 6.71 (s, 2H, Ar–H (mesitylene), 7.17–7.34 (m, 4H, Ar–H), 7.47–7.58 (m, 5H, Ar–H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 20.5, 21.4, 25.2, 39.1, 40.8, 115.4, 116.5, 127.1, 130.2, 130.3, 130.5, 132.5, 143.7, 150.9 (N–C–N), 151.8 (N–C–S), 160.8 (C–F), 205.3 (C=O). Elemental analysis: Calculated: C, 72.12; H, 6.05; N, 8.41; S, 6.42. Found: C, 72.25; H, 6.21; N, 8.32; S, 6.50.

Synthesis of 1-(3-methyl-3-mesityl)-cyclobutyl-2-{[5-(2-fluorophenyl)-4-benzyl-4H-1,2,4-triazol-3-yl] sulfanyl}-ethanone (6d) ($C_{31}H_{32}FN_3OS$)

White solid, yield 65%, m.p. 85–87 °C; FT-IR (KBr, cm^{-1}, υ): 2950–3065 (Ar–H), 2866–2925 (C–H), 1715 (C=O), 1434 (C=C), 1321 (C=N), 1220 (C–F), 1018 (N–N), 761 (C–S); ^{1}H-NMR (400 MHz, DMSO-d_6, δ, ppm): 1.49 (s, 3H, –CH_3 (cyclobutane)), 2.14 (s, 9H, –CH_3 (mesitylene)), 2.51–2.57 (m, 4H, –CH_2– (cyclobutane)), 3.55 (p, 1H, –CH– (cyclobutane), J = 8.70 Hz), 4.32 (s, 2H, S–CH_2–), 5.38 (s, 2H, N–CH_2–C_6H_5), 6.71 (s, 2H, Ar–H (mesitylene)), 7.17–7.34 (m, 4H, Ar–H), 7.47–7.58 (m, 5H, Ar–H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ, ppm): 20.5, 21.4, 25.2, 39.1, 40.0, 40.8, 48.1 115.4, 116.5, 125.2, 126.9, 130.5, 130.9, 132.5, 134.4, 134.9, 140.0, 143.7 (N–C–N), 151.9 (N–C–S), 160.8 (C–F), 205.3 (C=O). Elemental analysis: C, 72.48; H, 6.28; N, 8.18; S, 6.24. Found: C, 72.52; H, 6.35; N, 8.15; S, 6.30.

Biological assays

Antibacterial assays

The standard broth dilution method was used to measure the MIC values of the in vitro antimicrobial activities of our synthesized (6a–d) substances.[30] To examine the antibacterial activities, the synthesized compounds 6a–d and the control group were dissolved in DMSO (dimethyl sulfoxide). Furthermore, dilution series of decreasing concentrations of 1024, 512, 256, 128, 64, 32, 16, 8, 4 µg mL^{-1} were prepared for microorganisms at the indicated concentrations. Stock solutions were prepared in DMSO, and it was determined that DMSO did not have an effect on the microorganisms in the concentration. The microorganisms used were two Gram-positive bacteria and two Gram-negative bacteria. *B. subtilis* ATCC 6633, *L. monocytogenes* Scott A strains as Gram-positive bacteria, *P. vulgaris* FMC 1, and *S. typhimurium* NRRL B 4420 strains as Gram-negative bacteria were used. Ampicillin as a standard antibiotic was used for bacterial strains. Each bacterial strain in the stock was seeded in nutrient broth (pH 7.4) liquid under sterile conditions and allowed to incubate at 37 °C for 24 h. Thus, the concentration of bacterial strains on the media was adjusted to 105 CFU mL^{-1}. Test compounds dissolved in DMSO were first prepared at a concentration of 1024 µg mL^{-1} and then diluted up to 4 µg mL^{-1} by adding the medium. Besides this, a series of control groups were prepared. Bacterial cultures prepared one day in advance were inoculated in dilution tubes and allowed to incubate for 24 h at 37 °C. After incubation, MIC values were calculated using the turbidity determination method. Experiments were performed in two parallel runs.

Free radical clearing activity

The free radical scavenging activity of the compounds was determined with a small modification according to the Blois method using 1,1-diphenyl 2-picryl hydrazyl (DPPH).[32] The principle of the method is based on the reduction of 1,1-diphenyl 2-picrylhydrazyl radical (DPPH$^{\cdot}$), which is the colored free radical of free radical scavenger. DPPH is a stable red free radical. When free radicals are eliminated by antioxidant compounds, the color turns from red to yellow. The decrease in the absorbance at 517 nm of the reaction mixture indicates increased antioxidant activity in the free radical scavenging.

Reagents used

0.1 mM DPPH (prepared in ethanol); α-tocopherol 1 mg mL^{-1}; Butyl hydroxy anisole 1 mg/mL; DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging activity, butylhydroxytoluene (BHT), α-tocopherol, and ethyl alcohol were obtained from Merck or Aldrich. In the study, the synthesized compound was dissolved in ethyl alcohol as 1 mg mL^{-1}. The standard was dissolved in ethanol again to be 1 mg mL^{-1}. The test compound and standards were placed in test tubes at 50, 100, 250 µg mL^{-1}, respectively, and completed with pure ethanol to be at a total volume of 3 mL. 1 mL of the stock DPPH solution was then added to each sample tube. It was incubated in the dark at room temperature. After incubation, the absorbances at 517 nm (UV–Vis Spectrophotometry: Shimadzu UV-1700) were measured. As a control, 3 mL of ethanol and 1 mL of DPPH

solution were used. Reduced absorbance gives the free radical scavenging activity of the remaining amount of DPPH solution. The calculations for DPPH radical scavenging activity in the reaction medium were calculated according to the following formula.

$$\%\text{Free radical scavenging activity} = (A_0 - A_1/A_0) \times 100,$$

where A_0 is an absorbance of the control reaction and A_1 is an Absorbance of sample or standard.

Conclusions

In this study, we synthesized a new series of sulfanyl compounds containing triazole and cyclobutane ring. The synthesis compounds were confirmed by FT-IR, ^{1}H-, and ^{13}C NMR techniques. The novel sulfanyl compounds were obtained in moderate yields varying from 62 to 68%, which were more than expected. It was investigated whether the synthesized compounds had in vitro, antioxidant and antibacterial activities. Among various sulfanyl compound derivatives, the 6c-coded compound showed higher antioxidant activity than the other derivatives and the standard BHT compound. This compound has a good radical scavenging activity. As a result, it is thought that the newly synthesized class of compounds may contribute to the knowledge of the literature by the investigated biological activities.

Statistical analysis

All statistical analyses in this study were performed using the SPSS/PC package program. The data were expressed as mean ± standard error.

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