

## Synthesis and antioxidant and antimicrobial evaluation of novel 4-substituted-1*H*-1,2,4-triazole derivatives

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A series of 4-benzyl/phenyl-3-(1-methyl-1*H*-indole-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione (**4a,b**) and 2-{4-[benzyl/phenyl-5-(substitutedbenzylthio)]-4*H*-1,2,4-triazole-3-yl}-1-methyl-1*H*-indole derivatives (**5a-p**) were synthesized and evaluated for their *in vitro* scavenging of DPPH and superoxide radical, and lipid peroxidation inhibition effects as well as their antimicrobial properties. DPPH radical scavenging capacity was found to be equipotent with BHT and found in compounds containing 1,2,4-triazole-5(4*H*)-thione moiety (**4a,b**). With regard to antimicrobial properties, compound **5k** showed slight antimicrobial activity against all the test microorganisms.

**Key Words:** Indole, 1,2,4-triazole, DPPH, superoxide, lipid peroxidation, antimicrobial activity

### Introduction

Exogenous chemicals in food systems and endogenous metabolic processes in the human body produce highly reactive free radicals, particularly oxygen-derived free radicals. Reactive oxygen species (ROS) is a collective term that describes the chemical species that are formed upon incomplete reduction of oxygen and includes the superoxide anion ( $O_2^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), and the hydroxyl radical (HO). ROS are constantly generated in the human body and are involved in various physiologically important biological reactions.<sup>1-3</sup>

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Under physiological conditions, there is a balance between the production of reactive oxygen and a biological system's ability to detoxify the reactive intermediates. Oxidative stress occurs when the generation of ROS in a system exceeds the system's ability to eliminate them. Excessive generation of ROS induced by various stimuli leads to a variety of pathophysiological abnormalities such as inflammation, diabetes, genotoxicity, and cancer.<sup>4-6</sup> Treatment with antioxidants seemed to be a promising therapeutic approach for these conditions. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. Therefore, in recent years there has been a growing interest in developing new and efficient antioxidant compounds.<sup>7,8</sup>

Indoles are very common in the body and the diet, and synthetic and natural products containing indole moieties are an important class of therapeutic agents.<sup>9,10</sup> In recent decades, antioxidant activity of synthetic indole derivatives and their possible activity mechanisms have been studied. Indole structure, especially substitution of 2-position of indole, influences the antioxidant efficacy in biological systems.<sup>11</sup> *N*-Substituted indole-2/3-carboxamide and also ester derivatives show potent antioxidant activity against superoxide radical.<sup>12-14</sup> Inhibitory effect on lipid peroxidation of 2-phenyl indole derivatives was found as potent as butylatedhydroxytoluene (BHT) and compounds bearing electron-withdrawing groups showed the highest reduction in lipid peroxidation values.<sup>15</sup>

1,2,4-Triazole moiety has great versatility in fusing to various ring systems and possesses a broad spectrum of biological activities.<sup>16-19</sup> Antioxidant properties of 1,2,4-triazole derivatives have been investigated by employing various in vitro systems, i.e. interaction of 2,2-diphenyl-1-picrylhydrazyl (DPPH), scavenging of superoxide radical, and microsomal NADPH-dependent inhibition of lipid peroxidation (LP).<sup>20-23</sup> 1,2,4-Triazole derivatives have been found to have interactions with DPPH. Triazole-containing indole derivatives showed protective activity against the oxidative injury of the ischemic myocardium and they scavenged superoxide anion and hydroxyl radical.<sup>24</sup>

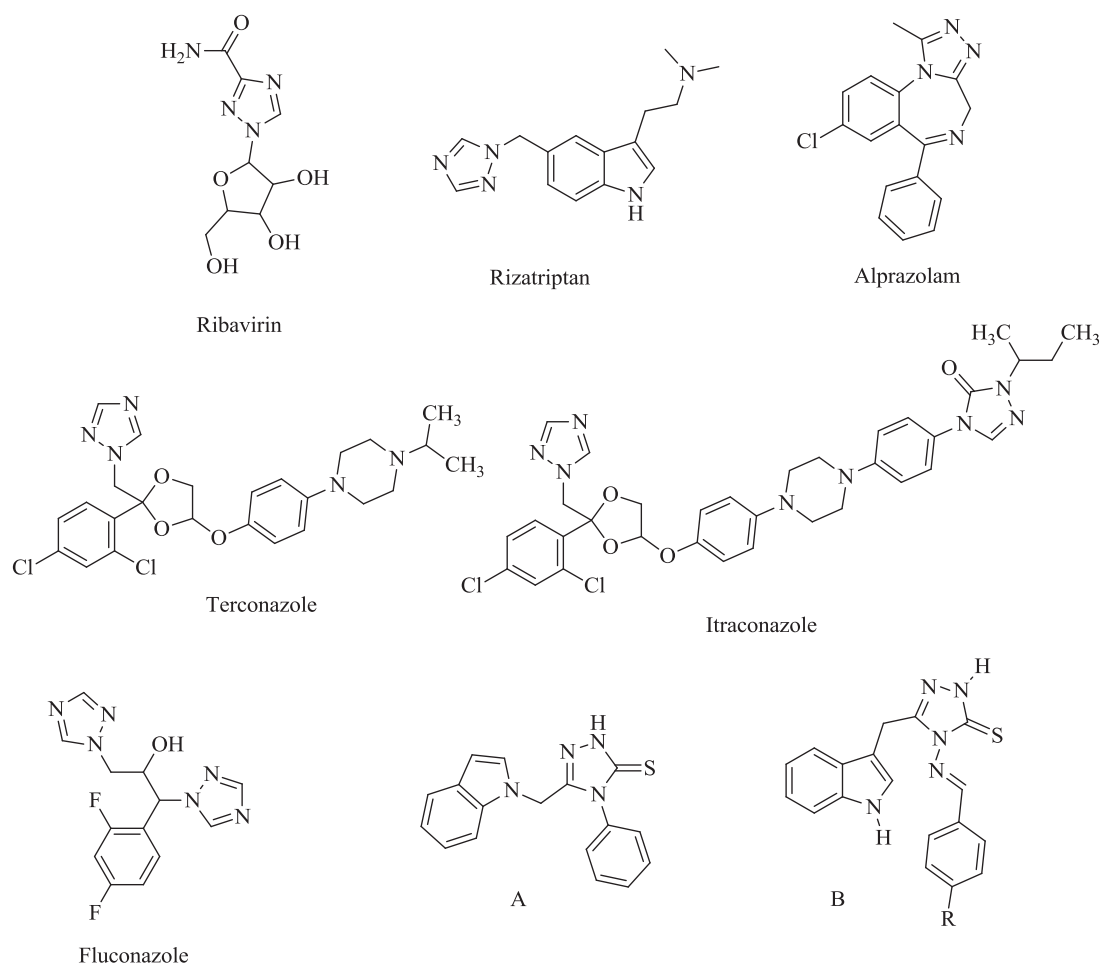
At the beginning of the twentieth century, infectious diseases were the leading cause of death worldwide. However, not only the emergence of new sources of infection but also developing resistance to antibiotics of existing infectious pathogens caused difficulties in the treatment of these diseases.<sup>25</sup>

New antibacterial agents to treat infections caused by these gram-positive bacteria have recently been introduced, including the semisynthetic streptogramins quinupristin/dalfopristin, daptomycin, the synthetic oxazolidinone linezolid and tigecycline.<sup>26</sup>

Some indole-containing compounds are known to have antibacterial activities.<sup>27</sup> Coumarin containing indole derivatives exhibited good to excellent in vitro activities against *Staphylococcus aureus* and *Enterococcus faecium* including drug-resistant gram-positive bacterial pathogens methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE).<sup>28</sup> Bis(indole) alkaloids were found to be important key structures for the treatment of *S. aureus* infections via inhibition of sortase A (SrtA) activity.<sup>27</sup> In addition, 1*H*-indole-4,7-dione derivatives showed potent antifungal activity against *Candida krusei*, *Candida neoformans*, and *Aspergillus niger*.<sup>29</sup> Substituted pyrazino[1,2-*a*]indole was found to have antibacterial activity against pathogenic strains of *S. aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Streptomyces thermonitrificans*, and *Escherichia coli*.<sup>30</sup> Moreover, indole-substituted 2,5-dihydro-1*H*-2,5-pyrrolediones were found to have antibacterial activity against resistant strains of *Mycobacterium smegmatis*, *S. aureus*, and some other gram-positive bacteria. It was found that the activity of compounds against *S. aureus* and *M. smegmatis* improves with increasing hydrophobic

properties as well as with hydrogen bond acceptors, depending on their distance from the indole-2-position.<sup>31</sup> Ethyl 6-bromo-5-hydroxy-1*H*-indole-3-carboxylate derivatives display a variety of biological effects, such as antiviral effect, immunostimulative effect, and interferon-induced activity. In support of these findings, ethyl 1*H*-indole-3-carboxylate derivatives were examined for their anti-HCV activities and found to be promising leads.<sup>32</sup>

The 1,2,4-triazole and its derivatives were reported to exhibit various pharmacological activities such as antimicrobial, analgesic, anti-inflammatory, anticancer, and antioxidant properties.<sup>33,34</sup> Some of the present day drugs such as ribavirin (antiviral agent), rizatriptan (antimigraine agent), alprazolam (anxiolytic agent), fluconazole, terconazole, and itraconazole (antifungal agents) are the best examples of potent molecules possessing a triazole nucleus (Figure 1).<sup>35</sup> Compounds containing indole and 1,2,4-triazole moieties as hybrid molecules including different pharmacophores were found to have antibacterial activity against *Bacillus subtilis* (MIK = 250 µg/mL) (A in Figure).<sup>36</sup> Moreover, some other indolic 1,2,4-triazole derivatives exhibited good to excellent in vitro activities against *Bacillus cereus* (MIK = 125 µg/mL) (B in Figure).<sup>37</sup>



**Figure.** Examples of drugs used in clinical treatment based on triazole and indole structure.

In view of the above observations, in this study, a series of indole derivatives containing 1,2,4-triazole moiety at the 2-position were prepared in order to evaluate their in vitro antioxidant properties by determining superoxide anion formation, DPPH free radical scavenging activity, and LP on rat liver homogenate. Antimicrobial activities of the title compounds were also tested using microdilution.

## Experimental

### Chemistry

The chemical reagents used in synthesis were purchased from Sigma (Germany) and Aldrich (US). Thin-layer chromatography (TLC) was performed on Merck 60F254 plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or charring Dragendorff reagent. Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected. Elemental analyses were performed with a LECO-932 (C, H, N, S-Elemental Analyzer) at the Faculty of Pharmacy, Ankara University. <sup>1</sup>H-NMR spectra were recorded in CDCl<sub>3</sub> or DMSO-*d*<sub>6</sub> on a Varian Mercury 400 MHz FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of the Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as  $\delta$  (ppm). IR spectra were obtained using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory. High resolution mass spectra data (HRMS) were collected using a Waters LCT Premier XE Mass Spectrometer operating in ESI (+) mode, also coupled to an AQUITY Ultra Performance Liquid Chromatography system.

#### Ethyl 1-methyl-1*H*-indole-2-carboxylate 1

Thionyl chloride (1.35 mL, 0.018 mol) was added dropwise with stirring to dry ethanol (25 mL) at -10 °C; indole-2-carboxylic acid 2.5 g (0.015 mol) was then added in one portion and the mixture was stirred for 30 min at 0 °C. After stirring for a further 1 h at room temperature, the mixture was heated to reflux for 4 h and evaporated. The white crystalline powder was dissolved in dichloromethane, extracted with 5% sodium hydrogen carbonate solution and dried over sodium sulfate. The product, ethyl 1*H*-indole-2-carboxylate, was filtered off and dried. Yield 2.77 g, (94.4%), mp 123 °C (Lit. mp 121-123 °C).<sup>38</sup> Ethyl 1*H*-indole-2-carboxylate (2.57 g, 0.013 mol) was dissolved in 25 mL of N,N-dimethylformamide and then (0.48 g, 0.02 mol) sodium hydride was added in an ice bath. Methyl iodide (125  $\mu$ L, 0.01 mol) was added at room temperature and stirred for 1 h. The reaction mixture was poured into ice-water. The crude product was filtered off and dried. Yield 2.11 g, (76.4%), mp 61 °C (Lit. mp 60-61 °C).<sup>38</sup>

#### 1-Methyl-1*H*-indol-2-carbohydrazide 2

Hydrazine hydrate (3 mL, 63 mmol) and ethyl 1-methyl-1*H*-indole-2-carboxylate (11.57 g, 8 mmol) in 20 mL of ethanol were refluxed for 6 h. The reaction mixture was cooled to room temperature and poured into water. The crude product was filtered off and dried. Yield 1.0 g, (68.5%), mp 158 °C (Lit. mp 156.2-157 °C).<sup>38</sup>

**General procedure for the preparation of *N*-substituted-2-(1-methyl-1*H*-indole-2-carbonyl)hydrazinecarbothioamide derivatives 3a,b**

1-Methyl-1*H*-indol-2-carbohydrazide 2 (6 mmol) in 35 mL of absolute ethanol and appropriate isothiocyanates (8 mmol) were heated under reflux for 3 h. The reaction mixture was cooled to room temperature and the precipitate formed was filtered off and recrystallized.

***N*-Benzyl-2-(1-methyl-1*H*-indole-2-carbonyl)hydrazinecarbothioamide 3a**

Recrystallized from ethanol as white solid (yield 2.12 g, 85%). mp 185-186 °C; IR (FTIR/FTNIR-ATR): 3174 (N-H), 2973 (C-H), 1671 (C=O), 1350 (C=S). <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 8.86 (bs, 1H, NH), 8.68 (bs, 1H, NH), 7.61 (d, 1H, *J* = 8.4 Hz, indole H<sub>4</sub>), 7.37-7.13 (m, 9H, ArH, NH), 7.07 (s, 1H, indole H<sub>3</sub>), 4.80 (d, 2H, *J* = 8 Hz, NH-CH<sub>2</sub>-phenyl), 3.85 (s, 3H, CH<sub>3</sub>). HRMS C<sub>18</sub>H<sub>18</sub>N<sub>4</sub>OS [M+H]<sup>+</sup> *Calc.* 339.1280, Found *m/z* 339.1263.

***N*-Phenyl-2-(1-methyl-1*H*-indole-2-carbonyl)hydrazinecarbothioamide 3b**

Recrystallized from ethanol as white solid (yield 1.68 g, 78.5%). mp 162-163 °C; IR (FTIR/FTNIR-ATR): 3132 (N-H), 2955 (C-H), 1666 (C=O), 1351 (C=S). <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 9.29 (bs, 1H, NH), 8.98 (bs, 1H, NH), 8.28 (s, 1H, NH), 7.66 (d, 1H, *J* = 8.4 Hz, indole H<sub>4</sub>), 7.41-7.15 (m, 8H, ArH), 7.14 (s, 1H, indole H<sub>3</sub>), 3.99 (s, 3H, CH<sub>3</sub>). HRMS C<sub>17</sub>H<sub>16</sub>N<sub>4</sub>OS [M+H]<sup>+</sup> *Calc.* 325.1123, Found *m/z* 325.1109. Anal. *Calc.* (%) for C<sub>17</sub>H<sub>15</sub>N<sub>4</sub>OS. C: 62.94 H: 4.97 N: 17.27, S: 9.72; Found C: 62.33 H: 5.17 N: 16.87 S: 9.74.

**General procedure for the preparation of 4-Benzyl/phenyl-3-(1-methyl-1*H*-indole-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thion derivatives 4a,b**

Appropriate carbothioamides (3 mmol) (3a,b) in 20 mL of 2 N sodium hydroxide were refluxed for 7 h. The reaction mixture was cooled and then acidified to pH 6 with 1 N hydrochloric acid. The precipitate was filtered, washed with water, and recrystallized from ethanol.

**4-Benzyl-3-(1-methyl-1*H*-indole-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione 4a**

White solid (yield 0.97 g, 96.6%). mp 236 °C; IR (FTIR/FTNIR-ATR): 3086 (N-H), 2940 (C-H), 1589-1508 (C=C), 1341 (C=S); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 11.34 (s, 1H, N-H), 7.61 (d, 1H, *J* = 8 Hz, indole H<sub>4</sub>), 7.34-7.17 (m, 6H, ArH), 7.10-7.07 (m, 2H, ArH), 6.61 (s, 1H, indole H<sub>3</sub>), 5.38 (s, 2H, N-CH<sub>2</sub>-phenyl), 3.55 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>18</sub>H<sub>16</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 321.1174; Found *m/z* 321.1178. Anal. *Calc.* (%) for C<sub>18</sub>H<sub>15</sub>N<sub>4</sub>S. C: 67.47 H: 5.03 N: 17.49, S: 9.87; Found C: 67.09 H: 4.96 N: 17.25 S: 9.88.

**4-Phenyl-3-(1-methyl-1*H*-indole-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione 4b**

White solid (yield 0.92 g, 88.2%). mp 280 °C; IR (FTIR/FTNIR-ATR): 3062 (N-H), 2914 (C-H), 1587-1511 (C=C), 1350 (C=S); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 11.32 (bs, 1H, N-H), 7.56-7.53 (m, 3H, indole H<sub>4</sub>, ArH), 7.42 (d, 1H, *J* = 8.4 Hz, indole H<sub>7</sub>), 7.38-7.27 (m, 4H, ArH), 7.08 (m, 1H, *J* = 7.6 Hz, ArH), 6.03 (s, 1H, indole H<sub>3</sub>), 3.96 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>17</sub>H<sub>14</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 307.1017, Found *m/z* 307.1019. Anal. *Calc.* (%) for C<sub>17</sub>H<sub>13</sub>N<sub>4</sub>S. C: 66.04 H: 4.61 N: 18.29, S: 10.30; Found C: 65.75 H: 4.68 N: 18.05 S: 10.33.

**General procedure for the preparation of 2-{4-[benzyl/phenyl-5-(substituted benzylthio)]-4H-1,2,4-triazole-3-yl}-1-methyl-1H-indole derivatives 5a-p**

4-Benzyl/phenyl-3-(1-methyl-1H-indole-2-yl)-1H-1,2,4-triazole-5(4H)-thione (0.8 mmol) in 20 mL of 2 N ethanolic sodium hydroxide and appropriate benzyl bromide were heated under reflux for 7 h. The precipitate formed was cooled to room temperature, filtered, and recrystallized from ethanol.

**2-{4-[Benzyl-5-(benzylthio)]-4H-1,2,4-triazole-3-yl}-1-methyl-1H-indole 5a**

White solid (yield 115.3 mg, 37%). mp 159-160 °C; IR (FTIR/FTNIR-ATR): 3027 (C-H), 2955 (C-H), 1589-1494 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.56 (d, 1H, *J* = 8.0 Hz, indole H<sub>4</sub>), 7.37 (d, 1H, *J* = 8.8 Hz, indole H<sub>7</sub>), 7.35-7.25 (m, 9H, ArH), 7.13 (m, 1H, ArH), 6.88-6.85 (m, 2H, ArH), 6.51 (s, 1H, indole H<sub>3</sub>), 5.05 (s, 2H, N-CH<sub>2</sub>-phenyl), 4.48 (s, 2H, S-CH<sub>2</sub>-phenyl), 3.79 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>25</sub>H<sub>22</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 411.1643, Found m/z 411.1630; Anal. *Calc.* (%) for C<sub>25</sub>H<sub>21</sub>N<sub>4</sub>S. C: 73.14 H: 5.40 N: 13.65, S: 7.63; Found C: 73.28 H: 5.19 N: 13.59 S: 7.84.

**2-{4-Benzyl-5-[(4-methylbenzyl)thio]-4H-1,2,4-triazole-3-yl}-1-methyl-1H-indole 5b**

White solid (yield 100 mg, 40%). mp 159-160 °C; IR (FTIR/FTNIR-ATR): 3023 (C-H), 1589-1512 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.56 (d, 1H, *J* = 8.0 Hz, indole H<sub>4</sub>), 7.37 (d, 1H, *J* = 8.8 Hz, indole H<sub>7</sub>), 7.32-7.22 (m, 6H, ArH), 7.15-7.13 (m, 2H, ArH), 7.11 (t, 1H, *J* = 7.6 Hz, ArH), 6.87 (m, 2H, ArH), 6.52 (s, 1H, indole H<sub>3</sub>), 5.07 (s, 2H, N-CH<sub>2</sub>), 4.45 (s, 2H, S-CH<sub>2</sub>), 3.80 (s, 3H, N-CH<sub>3</sub>), 2.33 (s, 3H, CH<sub>3</sub>-phenyl); HRMS C<sub>26</sub>H<sub>24</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 425.1800, Found m/z 425.1792; Anal. *Calc.* (%) for C<sub>26</sub>H<sub>23</sub>N<sub>4</sub>S. C: 73.55 H: 5.70 N: 13.20, S: 7.23; Found C: 73.66 H: 5.80 N: 13.13 S: 7.54.

**2-{4-Benzyl-5-[(2,6-dichlorobenzyl)thio]-4H-1,2,4-triazole-3-yl}-1-methyl-1H-indole 5c**

White solid (yield 154.3 mg, 41.2%). mp 129-130 °C; IR (FTIR/FTNIR-ATR): 3063 (C-H), 2934 (C-H), 1582-1562 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.58 (d, 1H, *J* = 8.0 Hz, indole H<sub>4</sub>), 7.38 (d, 1H, *J* = 8.4 Hz, indole H<sub>7</sub>), 7.33-7.26 (m, 6H, ArH), 7.19 (m, 1H, ArH), 7.14 (m, 1H, ArH), 6.92-6.89 (m, 2H, ArH), 6.55 (s, 1H, indole H<sub>3</sub>), 5.12 (s, 2H, N-CH<sub>2</sub>), 4.74 (s, 2H, S-CH<sub>2</sub>), 3.80 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>25</sub>H<sub>20</sub>Cl<sub>2</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 479.0864, Found m/z 479.0861; Anal. *Calc.* (%) for C<sub>25</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>4</sub>S. C: 62.63 H: 4.20 N: 11.69, S: 6.49; Found C: 62.54 H: 4.28 N: 11.68 S: 6.73.

**2-{4-Benzyl-5-[(4-bromobenzyl)thio]-4H-1,2,4-triazole-3-yl}-1-methyl-1H-indole 5d**

White solid (yield 144 mg, 37.7%). mp 148-149 °C; IR (FTIR/FTNIR-ATR): 2935 (C-H), 1583-1495 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.57 (d, 1H, *J* = 8.0 Hz, indole H<sub>4</sub>), 7.42 (d, 1H, *J* = 8.8 Hz, indole H<sub>7</sub>), 7.39-7.23 (m, 8H, ArH), 7.13 (m, 1H, ArH), 6.87-6.85 (m, 2H, ArH), 6.53 (s, 1H, indole H<sub>3</sub>), 5.12 (s, 2H, N-CH<sub>2</sub>), 4.43 (s, 2H, S-CH<sub>2</sub>), 3.81 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>25</sub>H<sub>21</sub>BrN<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 489.0749, Found m/z 489.0749; Anal. *Calc.* (%) for C<sub>25</sub>H<sub>20</sub>BrN<sub>4</sub>S. C: 61.35 H: 4.32 N: 11.45, S: 6.49; Found C: 61.47 H: 4.08 N: 11.38 S: 6.61.

**2-{4-Benzyl-5-[(3-methoxybenzyl)thio]-4H-1,2,4-triazole-3-yl}-1-methyl-1H-indole 5e**

White solid (yield 183.7 mg, 53.5%). mp 89-90 °C; IR (FTIR/FTNIR-ATR): 2935 (C-H), 1599-1584 (C=C), 1270 and 1040 (C-O-C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.57 (d, 1H, *J* = 8.0 Hz, indole H<sub>4</sub>), 7.37 (d, 1H, *J* = 8.4 Hz, indole H<sub>7</sub>), 7.32-7.20 (m, 6H, ArH), 7.15 (m, 1H, ArH), 6.93 (d, 1H, *J* = 7.6 Hz, ArH), 6.88 (m, 2H, ArH), 6.83 (m, 1H, ArH), 6.53 (s, 1H, indole H<sub>3</sub>), 5.06 (s, 2H, N-CH<sub>2</sub>-phenyl), 4.45 (s, 2H, S-CH<sub>2</sub>-phenyl), 3.79 (s, 3H, N-CH<sub>3</sub>), 3.77 (s, 3H, O-CH<sub>3</sub>). HRMS C<sub>26</sub>H<sub>24</sub>N<sub>4</sub>OS [M+H]<sup>+</sup> *Calc.* 441.1749, Found m/z 441.1743; Anal. *Calc.* (%) for C<sub>26</sub>H<sub>23</sub>N<sub>4</sub>OS. C: 70.88 H: 5.59 N: 12.72, S: 7.07; Found C: 70.60 H: 5.55 N: 12.69 S: 7.27.

**2-{4-Benzyl-5-[(4-methoxybenzyl)thio]-4H-1,2,4-triazole-3-yl}-1-methyl-1H-indole 5f**

White solid (yield 245.4 mg, 71.4%). mp 125-126 °C; IR (FTIR/FTNIR-ATR): 3033 (C-H), 2936 (C-H), 1607-1583 (C=C), 1251 and 1033 (C-O-C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.56 (d, 1H, *J* = 7.6 Hz, indole H<sub>4</sub>), 7.37 (d, 1H, *J* = 8.4 Hz, indole H<sub>7</sub>), 7.32-7.26 (m, 6H, ArH), 7.13 (t, 1H, *J* = 7.6 Hz, ArH), 6.88 (m, 2H, ArH), 6.29 (d, 2H, *J* = 8.8 Hz, ArH), 6.52 (s, 1H, indole H<sub>3</sub>), 5.09 (s, 2H, N-CH<sub>2</sub>), 4.45 (s, 2H, S-CH<sub>2</sub>), 3.80 (s, 3H, N-CH<sub>3</sub>), 3.79 (s, 3H, O-CH<sub>3</sub>). HRMS C<sub>26</sub>H<sub>24</sub>N<sub>4</sub>OS [M+H]<sup>+</sup> *Calc.* 441.1749, Found m/z 441.1760; Anal. *Calc.* (%) for C<sub>26</sub>H<sub>23</sub>N<sub>4</sub>OS. C: 70.88 H: 5.59 N: 12.72, S: 7.07; Found C: 70.76 H: 5.61 N: 12.61 S: 7.21.

**2-{4-Benzyl-5-[(4-(trifluoromethyl)benzyl)thio]-4H-1,2,4-triazole-3-yl}-1-methyl-1H-indole 5g**

White solid (yield 339.5 mg, 91%). mp 136-137 °C; IR (FTIR/FTNIR-ATR): 3033 (C-H), 2941 (C-H), 1615 and 1584 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.56 (d, 1H, *J* = 8.0 Hz, indole H<sub>4</sub>), 7.55-7.49 (m, 4H, ArH), 7.38 (d, 1H, *J* = 8.8 Hz, indole H<sub>7</sub>), 7.33-7.25 (m, 4H, ArH), 7.14 (m, 1H, ArH), 6.88 (m, 2H, ArH), 6.54 (s, 1H, indole H<sub>3</sub>), 5.14 (s, 2H, N-CH<sub>2</sub>), 4.53 (s, 2H, S-CH<sub>2</sub>), 3.82 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>26</sub>H<sub>21</sub>F<sub>3</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 479.1517, Found m/z 479.1525; Anal. *Calc.* (%) for C<sub>26</sub>H<sub>20</sub>F<sub>3</sub>N<sub>4</sub>S. C: 65.26 H: 4.42 N: 11.71, S: 6.49; Found C: 65.09 H: 4.25 N: 11.63, S: 6.73.

**2-{4-Benzyl-5-[(4-(trifluoromethoxy)benzyl)thio]-4H-1,2,4-triazole-3-yl}-1-methyl-1H-indole 5h**

White solid (yield 340.6 mg, 88.39%). mp 123-124 °C; IR (FTIR/FTNIR-ATR): 3037 (C-H), 2943 (C-H), 1596-1504 (C=C), 1255 and 1086 (C-O-C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.57 (d, 1H, *J* = 8.0 Hz, indole H<sub>4</sub>), 7.42-7.26 (m, 7H, ArH), 7.15 (m, 3H, ArH), 6.88 (m, 2H, ArH), 6.54 (s, 1H, indole H<sub>3</sub>), 5.13 (s, 2H, N-CH<sub>2</sub>-phenyl), 4.48 (s, 2H, S-CH<sub>2</sub>-phenyl), 3.82 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>26</sub>H<sub>21</sub>F<sub>3</sub>N<sub>4</sub>OS [M+H]<sup>+</sup> *Calc.* 495.1466, Found m/z 495.1471; Anal. *Calc.* (%) for C<sub>26</sub>H<sub>20</sub>F<sub>3</sub>N<sub>4</sub>OS. C: 63.15 H: 4.28 N: 11.33, S: 6.39; Found C: 63.20 H: 4.13 N: 11.42, S: 6.62.

**2-[5-(Benzylthio)-4-phenyl-4H-1,2,4-triazole-3-yl]-1-methyl-1H-indole 5i**

White solid (yield 231.4 mg, 72.7%). mp 156 °C; IR (FTIR/FTNIR-ATR): 3059 (C-H), 2949 (C-H), 1588-1578 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.51-7.44 (m, 3H, indole H<sub>4</sub>, ArH), 7.40-7.35 (m, 3H, indole H<sub>7</sub>, ArH), 7.31-7.24 (m, 5H, ArH), 7.14 (d, 2H, *J* = 7.6 Hz, ArH), 7.05 (m, 1H, ArH), 5.92 (s, 1H, indole H<sub>3</sub>), 4.52 (s, 2H, S-CH<sub>2</sub>), 4.12 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>24</sub>H<sub>20</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 397.1487, Found m/z 397.1485; Anal. *Calc.* (%) for C<sub>24</sub>H<sub>19</sub>N<sub>4</sub>S. C: 72.70 H: 5.08 N: 14.13, S: 8.02; Found C: 72.73 H: 4.97 N: 14.00, S: 8.13.

**2-{5-[(4-Methylbenzyl)thio]-4-phenyl-4*H*-1,2,4-triazole-3-yl}-1-methyl-1*H*-indole 5j**

White solid (yield 262.2 mg, 79.6%). mp 160-162 °C; IR (FTIR/FTNIR-ATR): 3048 (C-H), 1580-1512 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.53-7.35 (m, 4H, indole H<sub>4</sub>, H<sub>7</sub>, ArH), 7.28-7.23 (m, 4H, ArH), 7.15 (d, 2H, *J* = 6.8 Hz, ArH), 7.10 (d, 2H, *J* = 7.6 Hz, ArH), 7.05 (t, 1H, *J* = 7.2 Hz, ArH), 5.92 (s, 1H, indole H<sub>3</sub>), 4.50 (s, 2H, S-CH<sub>2</sub>), 4.12 (s, 3H, N-CH<sub>3</sub>), 2.31 (s, 3H, phenyl-CH<sub>3</sub>); HRMS C<sub>25</sub>H<sub>22</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 411.1643, Found *m/z* 411.1629; Anal. *Calc.* (%) for C<sub>25</sub>H<sub>21</sub>N<sub>4</sub>S. C: 73.14 H: 5.40 N: 13.65, S: 7.58; Found C: 73.41 H: 5.26 N: 13.54, S: 7.85.

**2-{5-[(2,6-dichlorobenzyl)thio]-4-phenyl-4*H*-1,2,4-triazole-3-yl}-1-methyl-1*H*-indole 5k**

White solid (yield 191 mg, 51%). mp 153-154 °C; IR (FTIR/FTNIR-ATR): 3092 and 3060 (C-H), 2958 (C-H), 1594-1582 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.52-7.36 (m, 5H, indole H<sub>4</sub>, ArH), 7.29-7.22 (m, 5H, indole H<sub>3</sub>, ArH), 7.15 (t, 1H, *J* = 8.0 Hz, ArH), 7.06 (m, 1H, ArH), 5.96 (s, 1H, indole H<sub>3</sub>), 4.81 (s, 2H, S-CH<sub>2</sub>), 4.13 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>24</sub>H<sub>18</sub>Cl<sub>2</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 465.0707, Found *m/z* 465.0701; Anal. *Calc.* (%) for C<sub>24</sub>H<sub>17</sub>Cl<sub>2</sub>N<sub>4</sub>S C: 61.04 H: 3.90 N: 12.04, S: 6.63; Found C: 61.24 H: 3.90 N: 12.84, S: 6.81.

**2-{5-[(4-Bromobenzyl)thio]-4-phenyl-4*H*-1,2,4-triazole-3-yl}-1-methyl-1*H*-indole 5l**

White solid (yield 292.3 mg, 76.5%). mp 185-186 °C; IR (FTIR/FTNIR-ATR): 3060 (C-H), 2951 (C-H), 1587-1578 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.55-7.35 (m, 6H, indole H<sub>4</sub>, H<sub>7</sub>, ArH), 7.31-7.24 (m, 4H, ArH), 7.17 (d, 2H, *J* = 8.0 Hz, ArH), 7.05 (m, 1H, ArH), 5.92 (s, 1H, indole H<sub>3</sub>), 4.46 (s, 2H, S-CH<sub>2</sub>), 4.11 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>24</sub>H<sub>19</sub>BrN<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 475.0592, Found *m/z* 475.0595; Anal. *Calc.* (%) for C<sub>24</sub>H<sub>18</sub>BrN<sub>4</sub>S C: 60.63 H: 4.03 N: 11.79, S: 6.52; Found C: 60.36 H: 3.88 N: 11.61, S: 6.78.

**2-{5-[(3-Methoxybenzyl)thio]-4-phenyl-4*H*-1,2,4-triazole-3-yl}-1-methyl-1*H*-indole 5m**

White solid (yield 288.3 mg, 84.2%). mp 156-157 °C; IR (FTIR/FTNIR-ATR): 3100 (C-H), 2945 (C-H), 1598-1578 (C=C), 1271 and 1035 (C-O-C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.51-7.45 (m, 3H, indole H<sub>4</sub>, ArH), 7.40 (dd, 2H, *J* = 12.8 Hz, *J* = 13.2 Hz, indole H<sub>4</sub>, ArH), 7.27 (m, 1H, indole H<sub>7</sub>), 7.20 (m, 1H, ArH), 7.14 (d, 2H, *J* = 7.2 Hz, ArH), 7.05 (m, 1H, ArH), 6.93 (d, 1H, *J* = 7.2 Hz, ArH), 6.90 (s, 1H, ArH), 6.81 (d, 1H, *J* = 8.4 Hz, ArH), 5.93 (s, 1H, indole H<sub>3</sub>), 4.49 (s, 2H, S-CH<sub>2</sub>), 4.11 (s, 3H, N-CH<sub>3</sub>), 3.77 (s, 3H, O-CH<sub>3</sub>); HRMS C<sub>25</sub>H<sub>22</sub>N<sub>4</sub>OS [M+H]<sup>+</sup> *Calc.* 427.1593, Found *m/z* 427.1573; Anal. *Calc.* (%) for C<sub>25</sub>H<sub>21</sub>N<sub>4</sub>OS C: 70.40 H: 5.20 N: 13.14 S: 7.43; Found C: 70.66 H: 5.12 N: 13.10 S: 7.59.

**2-{5-[(4-Methoxybenzyl)thio]-4-phenyl-4*H*-1,2,4-triazole-3-yl}-1-methyl-1*H*-indole 5n**

White solid (yield 201.2 mg, 58.8%). mp 146-147 °C; IR (FTIR/FTNIR-ATR): 3100 (C-H), 2931 (C-H), 1608 and 1582 (C=C), 1247 and 1031 (C-O-C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.51-7.44 (m, 3H, indole H<sub>4</sub>, ArH), 7.38 (d, 2H, *J* = 12.0 Hz, ArH), 7.31 (d, 1H, *J* = 8.4 Hz, indole H<sub>7</sub>), 7.25 (m, 2H, ArH), 7.17 (d, 2H, *J* = 8.0 Hz, ArH), 7.05 (m, 1H, ArH), 6.82 (d, 2H, *J* = 8.4 Hz, ArH), 5.93 (s, 1H, indole H<sub>3</sub>), 4.49 (s, 2H, S-CH<sub>2</sub>), 4.12 (s, 3H, N-CH<sub>3</sub>), 3.78 (s, 3H, O-CH<sub>3</sub>); HRMS C<sub>25</sub>H<sub>22</sub>N<sub>4</sub>OS [M+H]<sup>+</sup> *Calc.* 427.1593, Found *m/z* 427.1595; Anal. *Calc.* (%) for C<sub>25</sub>H<sub>21</sub>N<sub>4</sub>OS C: 70.40 H: 5.20 N: 13.14 S: 7.43; Found C: 70.28 H: 5.04 N: 13.03 S: 7.46.



**2-{5-[(4-(Trifluoromethyl)benzyl)thio]-4-phenyl-4*H*-1,2,4-triazole-3-yl]-1-methyl-1*H*-indole 5o**

White solid (yield 232.8 mg, 62.4%). mp 134-135 °C; IR (FTIR/FTNIR-ATR): 3055 (C-H), 2946 (C-H), 1578-1496 (C=C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.55-7.45 (m, 6H, indole H<sub>4</sub>, H<sub>7</sub>, ArH), 7.38 (dd, 2H, *J* = 10.8 Hz, *J* = 10.4 Hz, ArH), 7.25 (m, 2H, ArH), 7.17 (d, 2H, *J* = 8.0 Hz, ArH), 7.05 (t, 1H, *J* = 7.6 Hz, ArH), 5.92 (s, 1H, indole H<sub>3</sub>), 4.55 (s, 2H, S-CH<sub>2</sub>), 4.12 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>25</sub>H<sub>19</sub>F<sub>3</sub>N<sub>4</sub>S [M+H]<sup>+</sup> *Calc.* 465.1361, Found *m/z* 465.1345; Anal. *Calc.* (%) for C<sub>25</sub>H<sub>18</sub>F<sub>3</sub>N<sub>4</sub>S C: 64.64 H: 4.12 N: 12.06 S: 6.88; Found C: 64.61 H: 4.24 N: 12.01 S: 6.94.

**2-{5-[(4-(Trifluoromethoxy)benzyl)thio]-4-phenyl-4*H*-1,2,4-triazole-3-yl]-1-methyl-1*H*-indole 5p**

White solid (yield 287 mg, 74.4%). mp 117-118 °C; IR (FTIR/FTNIR-ATR): 3062 (C-H), 1577-1509 (C=C), 1248 and 1100 (C-O-C); <sup>1</sup>H-NMR (CDCl<sub>3</sub>); δ 7.53-7.43 (m, 4H, indole H<sub>4</sub>, H<sub>7</sub>, ArH), 7.38 (dd, 2H, *J* = 11.2 Hz, *J* = 11.6 Hz, ArH), 7.25 (m, 2H, ArH), 7.15 (m, 4H, ArH), 7.05 (t, 1H, *J* = 8.0 Hz, ArH), 5.93 (s, 1H, indole H<sub>3</sub>), 4.55 (s, 2H, S-CH<sub>2</sub>), 4.12 (s, 3H, N-CH<sub>3</sub>); HRMS C<sub>25</sub>H<sub>19</sub>F<sub>3</sub>N<sub>4</sub>OS [M+H]<sup>+</sup> *Calc.* 481.1310, Found *m/z* 481.1293; Anal. *Calc.* (%) for C<sub>25</sub>H<sub>18</sub>F<sub>3</sub>N<sub>4</sub>S C: 62.49 H: 3.99 N: 11.66 S: 6.36; Found C: 62.32 H: 4.13 N: 11.54 S: 6.69.

**Antioxidant and radical scavenging properties**

All synthesized indole 1,2,4-triazole derivatives were tested for DPPH, superoxide radical scavenging, and anti-LP activities. All the results were compared with BHT.

**Superoxide radical scavenging assay**

Superoxide was generated by xanthine/xanthine oxidase and measured by the inhibition of cytochrome c reduction as described by McCord and Fridovich with some modification.<sup>39</sup> The incubation mixture contained 100 μL of 4 mM xanthine, 400 μL of cytochrome c, 50 mL of 50 mM phosphate buffer (pH 7.8, 1 mM EDTA), and 10 mL of the test compounds were prepared in a 96-well plate, and then 40 μL of xanthine oxidase was added to each well. The absorbance of each reaction mixture was monitored at 550 nm for 3 min. Each experiment was triplicated.

$$\text{Superoxide radical scavenging activity (\%)} = (A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}} - A_{\text{blank}}) \times 100$$

(A<sub>control</sub> = the absorbance of the control, A<sub>sample</sub> = the absorbance of test sample A<sub>blank</sub> = the absorbance of blank)

**DPPH radical scavenging assay**

The DPPH assay was performed using test compounds, purified isolates as previously described.<sup>40</sup> DPPH reacts with antioxidant compounds. It is reduced, and then its deep violet color in methanol is bleached to yellow, showing a significant absorption decrease at 517 nm. According to the literature, test samples were dissolved in DMSO and mixed with methanol solutions of DPPH (100 mM) in 96-well plates. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. For each test compound,

different concentrations were tested. The antioxidant capacity of each test compound was expressed as an IC<sub>50</sub> value ± SD, i.e. the concentration in mM that inhibits DPPH absorption by 50%, and was calculated by linear regression analysis. Tests were carried out in triplicate. Free radical DPPH inhibition as a percentage was calculated as follows:

$$\text{Free radical DPPH inhibition (\%)} = [(A_0 - A_1/A_0) \times 100]$$

(A<sub>0</sub> = the absorbance of the control; A<sub>1</sub> = the absorbance of the sample)

## Assay of lipid peroxidation

The lipid peroxidation assay was performed according to the method of Mihara et al.<sup>41</sup> with some modification. Throughout the experiments, the animals were treated under the audit of Gazi University's Commission of Animal Ethics according to the suggested international ethical guidelines for the care of laboratory animals (Permission No: 10.046). Wistar rats (200-225 g) were fed standard laboratory rat chow and tap water ad libitum. The animals were fasted for 24 h and then sacrificed by decapitation under anesthesia. Their livers were removed, washed in ice-cold distilled water, and homogenized straight away with a Teflon homogenizer. LP was measured spectrophotometrically by estimation of thiobarbituric acid reactive substances (TBARS). Then 0.05 mL of different concentrations of synthesized compounds were incubated for 1 h at 37 °C in an assay mixture that contained 0.5 mL of liver homogenate, 0.1 mL of Tris-HCl buffer (pH 7.2), 0.05 mL of 0.1 mM ascorbic acid, and 0.05 mL of 4 mM FeCl<sub>2</sub>. After incubation, 3.0 mL of H<sub>3</sub>PO<sub>4</sub> and 1 mL of 0.6% TBA were added to the assay mixture. The prepared mixture was shaken vigorously, boiled for 30 min, and then cooled. n-Butanol was added and then the absorbance of the supernatant was read at 532 nm against a blank, which contained all reagents except liver homogenate. The difference was regarded as the TBA value. Lipid peroxidation inhibitory activity (%) is expressed as follows:

$$\text{Lipid peroxidation inhibitory activity (\%)} = (A_{\text{control}} - A_{\text{sample}})/(A_{\text{control}} - A_{\text{blank}}) \times 100$$

(A<sub>control</sub> = the absorbance of the control; A<sub>sample</sub> = the absorbance of the sample; A<sub>blank</sub> = the absorbance of the blank)

## Microbiology

Mueller Hinton Agar (MHA) (Merck), Mueller Hinton Broth (MHB) (Merck), Sabouraud Dextrose Agar (SDA) (Merck), RPMI-1640 medium with L-glutamine (Sigma), 3-[N-morpholino]-propanesulfonic acid (MOPS) (Sigma), 96-well microplates (Falcon), transfer pipette (Socorex), gentamicin (Sigma), fluconazole (Nobel), and dimethylsulfoxide (DMSO) (Riedel de Haen) were used in the antimicrobial activity testing.

*P. aeruginosa* ATCC 27853 (American Type Culture Collection), *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Candida albicans* ATCC 90028, and *Candida glabrata* RSKK 90018 (Refik Saydam Culture Collection) were included in the study.

Standard powders of gentamicin and fluconazole were obtained from the manufacturers. Stock solutions were dissolved in distilled water (gentamicin and fluconazole). All bacterial isolates were subcultured in MHA plates and incubated overnight at 37 °C and all *Candida* isolates were subcultured in SDA plates at 35 °C for 24-48 h. The microorganisms were passaged at least twice to ensure purity and viability.

The solutions of the newly synthesized compounds were prepared at 1256, 625, 312.5, 156.25, 78.125, 39.06, 19.5, and 9.76  $\mu\text{g}/\text{mL}$  concentrations by diluting in MHB.

Bacterial susceptibility testing was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) M100-S16.<sup>42</sup> The bacterial suspensions used for inoculation were prepared at  $10^5$  CFU/mL by diluting fresh cultures at McFarland 0.5 density ( $10^7$  CFU/mL). Suspensions of the bacteria at  $10^5$  CFU/mL concentration were inoculated to the 2-fold diluted solution of the compounds. There were  $10^4$  CFU/mL bacteria in the wells after inoculations. MHB was used for diluting the bacterial suspension and for 2-fold dilution of the compound. DMSO, standard antimicrobials, pure microorganisms, and pure media were used as control wells. Bacterial inoculum was added to each well of the microdilution trays. The trays were incubated at 37 °C in a humid chamber and MIC endpoints were read after 24 h of incubation. All organisms were tested in triplicate in each run of experiments. The lowest concentration of the compound that completely inhibited macroscopic growth was determined and minimum inhibitory concentrations (MICs) are noted in Table 3.

All *Candida* isolates were subcultured in SDA plates, incubated at 35 °C for 24-48 h prior to antifungal susceptibility testing, and passaged at least twice to ensure purity and viability. Susceptibility testing was performed in RPMI-1640 medium with L-glutamine buffered pH 7 with MOPS and culture suspensions were prepared through the guideline of CLSI M27-A.<sup>43</sup> The yeast suspensions used for inoculation were prepared at  $10^4$  cfu/mL by diluting fresh cultures at McFarland 0.5 density ( $10^6$  CFU/mL). Suspensions of the yeast at  $10^4$  CFU/mL concentration were inoculated to 2-fold diluted solution of the compounds. There were  $10^3$  CFU/mL bacteria in the wells after inoculations. Yeast inoculum was added to each well of the microdilution trays. The trays were incubated at 35 °C in a humid chamber and MIC endpoints were read after 48 h of incubation. All organisms were tested in triplicate in each run of experiments. The lowest concentration of the compound that completely inhibited macroscopic growth was determined and MICs are reported in Table 3.

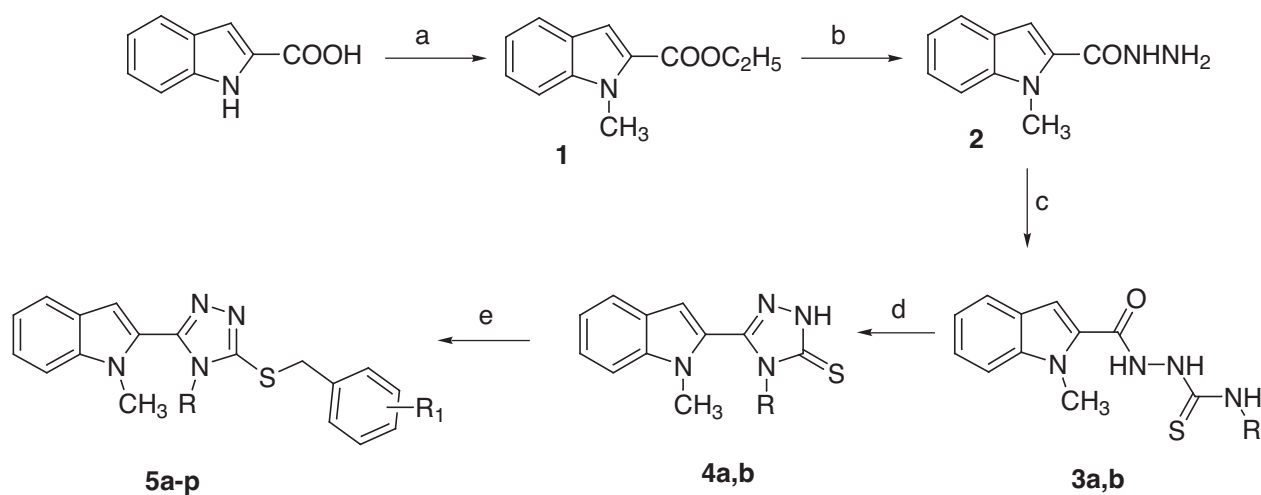
## Results and discussion

### Chemistry

The synthetic route for the newly synthesized compounds, 1,2,4-triazolyindole derivatives (**4a,b**; **5a-p**), is illustrated and outlined in the Scheme. Ethyl 1-methyl-1*H*-indole-2-carboxylate **1** was synthesized according to the literature.<sup>44</sup> Ester **1** was converted to the corresponding acid hydrazides **2** by refluxing with hydrazine hydrate (80%) in ethanol.<sup>45–48</sup> Hydrazinecarbothioamide derivatives **3a,b** were synthesized by the condensation of acid hydrazide **2** and benzyl/phenylisothiocyanates in ethanol. These compounds were used as the key intermediates for the synthesis of 1,2,4-triazole derivatives. The 4-substituted-3-(1-methyl-1*H*-indole-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thions **4a,b** were synthesized by intramolecular dehydrative cyclization of carbothioamides **3a,b** refluxed in 4 N sodium hydroxide solution, followed by neutralization with concentrated HCl.<sup>49</sup> 3-Alkylthio derivatives of **4a,b** were synthesized in yields ranging between 37% and 91% by the action of several benzyl chlorides on 4-substituted-3-(1-methyl-1*H*-indole-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thione **4a,b** in the presence of strong alkali. All the reactions were monitored by TLC. The structure elucidations of the newly synthesized compounds were carried out by spectroscopic techniques like IR and <sup>1</sup>H-NMR (Table 1). Further confirmations of the compounds were carried out by mass spectrometry and microanalysis.

The IR spectra of *N*-benzyl/phenyl-2-(1-methyl-1*H*-indole-2-carbonyl) hydrazinecarbothioamide derivatives **3a,b** exhibited characteristic strong absorption bands for carbonyl group at 1671-1666 cm<sup>-1</sup>, and C=S stretching bands at 1351-1350 cm<sup>-1</sup>. The C=S stretching bands of compounds **4a,b** appeared in the 1350-1341 cm<sup>-1</sup> region. These bands were not detectable for compounds **5a-p**, demonstrating the disappearance of this group.

The <sup>1</sup>H-NMR spectra of compounds **3a,b** displayed 3 singlets due to 3 different -NH groups in the δ 9.29-8.28 ppm range each showing the integration for 1 proton. In the <sup>1</sup>H-NMR spectra of compounds **4a,b**, signals of NH of the 1,2,4-triazole-5(4*H*)-thione derivatives were observed in the δ 11.34-11.32 ppm range as singlets. In the <sup>1</sup>H-NMR spectra of **5a-p**, the lack of signals arising from triazole CS-NH function at 11.34-11.32 ppm, observed with **4a,b**, provided the evidence for the formation of thioether function. Further support for the structures of these compounds was obtained through the presence of resonances arising from benzylic protons at 4.81-4.45 ppm. Further spectroscopic details of these compounds are presented in the experimental part.

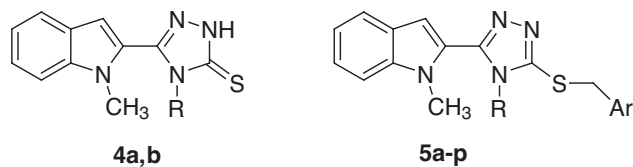


**Scheme.** Reagent and conditions a) i. SOCl<sub>2</sub>, EtOH, -10 °C; ii. CH<sub>3</sub>I, NaH, DMF, 0 °C - rt; b) NH<sub>2</sub>NH<sub>2</sub>, EtOH, reflux, 2 h; c) RNCS, EtOH; d) NaOH, H<sub>2</sub>O, reflux; e) Ar-CH<sub>2</sub>-Cl, 2N NaOH, EtOH, reflux, 7 h.

## Pharmacology

### Antioxidant activities

The compounds reported herein were tested for their scavenging effects of DPPH and superoxide radical, and their ability to inhibit lipid peroxidation (Table 2). Each method is related to the generation of a different radical acting through a variety of mechanisms.

**Table 1.** Synthesized indolic compounds containing 1,2,4-triazole-5(4H)-thione moiety.


Compound	R	Ar
4a	benzyl	
4b	phenyl	
5a	benzyl	
5b	benzyl	
5c	benzyl	
5d	benzyl	
5e	benzyl	
5f	benzyl	
5g	benzyl	
5h	benzyl	
5i	phenyl	
5j	phenyl	
5k	phenyl	
5l	phenyl	
5m	phenyl	
5n	phenyl	
5o	phenyl	
5p	phenyl	

The scavenging effects of synthesized compounds on DPPH radical are presented in Table 2. Preliminary screenings of the title compounds were performed at different concentrations to determine the scavenging effects on the DPPH. Compounds **4a** (IC<sub>50</sub> 90 µM) and **4b** (IC<sub>50</sub> 65 µM), derivatives with a free 1,2,4-triazole-5(4*H*)-thione ring, were found to be good scavengers of DPPH radical. In these conditions the reference drug BHT showed better scavenging effects on the DPPH. Compound **5h** bearing p-OCF<sub>3</sub> substitution on the S-benzyl ring exhibited a moderate effect on DPPH radical with an IC<sub>50</sub> value of 0.91 mM. Although the scavenging rates were not very pronounced, the compounds **5i**, **5k**, **5m**, and **5n** demonstrated a scavenging effect in the range of 5%-20%. The rest of the compounds had no effect on DPPH radical (Table 2).

The superoxide radical scavenging activities of the compounds were also investigated by using the xanthine/xanthine oxidase system, and there was no significant activity pattern obtained from this experiment (data are not shown).

**Table 2.** Effects of the compounds on LP levels and DPPH radical scavenging capacity.<sup>a</sup>

Compound	DPPH radical scavenging capacity		Lipid peroxidation inhibition % (1 mM)
	Inhibition% (1 mM)	(IC <sub>50</sub> mM)	
<b>4a</b>	<b>87 ± 3</b>	<b>0.09 ± 0.05</b>	<b>12 ± 2.4</b>
<b>4b</b>	<b>88 ± 4</b>	<b>0.065 ± 0.019</b>	<b>22 ± 2.3</b>
<b>5a</b>	NA		NA
<b>5b</b>	NA		NA
<b>5c</b>	NA		NA
<b>5d</b>	NA		NA
<b>5e</b>	NA		NA
<b>5f</b>	NA		NA
<b>5g</b>	NA		NA
<b>5h</b>	<b>61 ± 4</b>	<b>0.91 ± 0.1</b>	<b>11 ± 1.5</b>
<b>5i</b>	<b>4.5 ± 1</b>		NA
<b>5j</b>	NA		NA
<b>5k</b>	<b>5.0 ± 0.5</b>		NA
<b>5l</b>	NA		NA
<b>5m</b>	<b>10 ± 1</b>		<b>8 ± 1.2</b>
<b>5n</b>	<b>20 ± 2</b>		<b>17 ± 2.1</b>
<b>5o</b>	NA		NA
<b>5p</b>	NA		NA
<b>BHT</b>	98 ± 2	0.054 ± 0.005	65 ± 1.4

<sup>a</sup>Each value represents mean ± SD of 2-4 independent experiments. NA: not active

The inhibitory effect of the compounds on LP levels was examined by measuring the formation of 2-thiobarbituric acid reactive substance. As can be seen from Table 2, the BHT inhibited LP levels by about 65% at 1 mM concentration. The compounds **4a**, **4b**, **5h**, **5m**, and **5n** showed inhibitory properties on lipid

peroxidation level at the same concentration. Nonsubstituted 1,2,4-triazole-5(4*H*)-thione ring (compounds **4a** and **4b**) improved inhibitory action. The most active compound is N-phenyl-3-(1-methyl-1*H*-indole-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thion **4b**; it caused 22% inhibition on LP. Compounds **5h**, **5m**, and **5n** showed weak inhibitory effect in the range of 11%-17%. The other compounds had no inhibitory effect on LP levels.

Many studies have established beneficial effects of antioxidants that reduce the myocardial infarct size by interventions, which either attenuate the generation or reduce the effects of reactive oxygen species.<sup>50,51</sup> Andreadou et al. have reported on the synthesis and pharmacological properties of some indolic compounds bearing triazole moiety. They found that 3-[(1*H*-indolyl)methyl]-4-amino-4,5-dihydro-1*H*,1,2,4-triazole-5-thione has shown significant antioxidant properties by inhibiting in vitro non-enzymatic rat hepatic microsomal LP levels.<sup>52</sup> In our study, 4-benzyl/phenyl-3-(1-methyl-1*H*-indole-2-yl)-1*H*-1,2,4-triazole-5(4*H*)-thion derivatives **4a** and **4b** showed better antioxidant properties than their S-alkylated derivatives. The introduction of benzyl and phenyl rings in the 4th position of the 1,2,4-triazole did not significantly alter the activity. Therefore, the important structural feature for DPPH radical scavenging is the presence of the free 1,2,4-triazole-5(4*H*)-thione ring in the tested indolic compounds.

**Table 3.** Antimicrobial activities of the title compounds ( $\mu\text{g/mL}$ ).

Compound	Pa	Ec	Sa	Ef	Ca	Cg
<b>4a</b>	625	625	NI	NI	625	625
<b>4b</b>	625	NI	NI	NI	NI	625
<b>5a</b>	625	625	625	625	NI	NI
<b>5b</b>	625	625	NI	625	625	625
<b>5c</b>	625	625	NI	NI	625	625
<b>5d</b>	625	NI	NI	NI	625	625
<b>5e</b>	625	625	NI	625	NI	NI
<b>5f</b>	625	625	NI	625	625	NI
<b>5g</b>	625	625	625	625	625	NI
<b>5h</b>	625	625	625	625	NI	NI
<b>5i</b>	625	625	NI	625	NI	625
<b>5j</b>	625	NI	NI	NI	625	625
<b>5k</b>	625	625	625	625	625	625
<b>5l</b>	625	625	NI	NI	625	625
<b>5m</b>	625	625	625	625	NI	625
<b>5n</b>	625	625	625	625	NI	625
<b>5o</b>	625	625	NI	625	NI	NI
<b>5p</b>	625	625	625	625	NI	NI
Gentamicin	2	2	2	2	—	—
Fluconazole	—	—	—	—	64	64

Abbreviations: Pa, *P. aeruginosa* ATCC 27853; Ec, *E. coli* ATCC 25922; Sa, *S. aureus* ATCC 25923; Ef, *E. faecalis* ATCC 29212; Ca, *C. albicans* ATCC 90028; Cg, *Candida glabrata* RSKK 90018; NI: no inhibition

The relationships between the activities, the type of substituents, and their position in the title compounds were evaluated. Introducing benzyl moiety to the triazole portion of the title compounds resulted in inactive antioxidant compound. On the other hand, the presence of a methoxy group (compound **5m** and **5n**) in the aromatic ring of S-benzylated derivatives showed a more positive effect on the inhibition of LP than the methyl or halogen substituted compounds. It was found that p-OCF<sub>3</sub> substitution on the S-benzyl ring (compound **5h**) had a positive effect on DPPH radical scavenging capacity and the inhibition of LP.

## Microbiology

All compounds were evaluated for their antimicrobial properties. MICs were recorded as the minimum concentration of compound that inhibits the growth of the tested microorganisms. The MIC values were determined by a microdilution method in MHB and RPMI-1640 medium for the antibacterial and antifungal assays, respectively. According to the results, the compounds showed poor activity compared with gentamicin and fluconazole. According to the data (Table 3), all tested compounds were weakly effective against gram-negative *P. aeruginosa*. Compounds **4a** and **4b** were more effective against gram-negative bacteria and fungi than against gram-positive bacteria. Compounds **5a**, **5h**, and **5p** showed better activity against the tested bacteria than fungi. Compound **5k** showed slight antimicrobial activity against all the test microorganisms.

## Conclusions

We studied the antioxidative capacity of the synthesized compounds by determining superoxide (O<sub>2</sub><sup>•-</sup>) and DPPH radical scavenging capacity and the inhibition of LP. The results of the biological evaluation revealed that especially compounds **4a,b** exhibited good scavenging effects on DPPH radical. Evaluation of activity results for scavenger effect on DPPH and inhibition of LP suggested that the free 1,2,4-triazole-5(4*H*)-thione ring might be important for the activity. We think that the preliminary in vitro activity results of this class of compounds might lead to further studies to develop better candidates possessing antioxidant activity. Antimicrobial evaluations of some of the presented structures against bacteria and fungi are encouraging since compound **5k** showed slight antimicrobial activity against all the test microorganisms. It is considered that the solubility problem caused the generation of ineffective compounds. Designing more polar compounds may overcome solubility problems, and might achieve more active compounds. Further studies with these types of compounds are under investigation in our laboratory.

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