



ORIGINAL ARTICLE

Elucidation of potential anticancer, antioxidant and antimicrobial properties of some new triazole compounds bearing pyridine-4-yl moiety and cyclobutane ring



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KEYWORDS

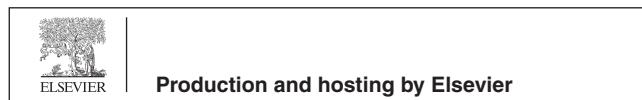
1,2,4-Triazole;
Anticancer;
Antioxidant;
Antimicrobial;
Biological activities

Abstract Thiosemicarbazides (2a–e) were obtained by the interaction of pyridine-4-carboxylic acid hydrazide (1) with five different isothiocyanate (RNCS) derivatives. By addition of KOH to the reaction medium, ethyl, allyl, phenyl and benzyl, p-tolyl substituted 1,2,4-triazoles (3a–e) were obtained. 3a–e were dissolved in dry acetone containing K₂CO₃ in the presence of 2-chloro-1-(3-methyl-3-mesitylcyclobutyl) ethanone (4) to give 3,4,5-trisubstituted 1,2,4-triazole sulfanyl compounds containing a cyclobutane ring (5a–e). The structures of the final compounds were confirmed by elemental analyses, FT-IR, ¹H NMR and ¹³C NMR. Due to many activities regarding pharmacology, Compounds such as 1,2,4-triazole attract high attention in the fields of chemistry, pharmacology, and biology. All the newly synthesised compounds were screened for their Antioxidant, antimicrobial, anti-cancer activities. The measurement of the antioxidant feature of the compounds was performed by DPPH radical scavenging activity technique. It was observed that the activity of some of the compounds was close to the standard antioxidant BHT. Furthermore, the anticancer effects of compounds were investigated against HT29 human colon adenocarcinoma cells. It has been noted that all -compounds inhibited cancerous cells in a statistically significant compared to the control. For the measurement of antimicrobial activity Agar well diffusion method was preferred. For the antimicrobial study, *S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli*, and a single fungi *C. albicans* have been used. They could also be stated as ATCC 29213, 29212, 27853, 2592, and

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10231 respectively. The resulting range of MIC values for the chemicals was between 15.625 - > 125 μM . In conclusion; all compounds have shown various and important bioactivities at different levels, such as being antioxidant, antimicrobial, and anticancer.

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1. Introduction

In recent years, a significant increase has been experienced in terms of the number of compounds with biological activities (Del-Toro-Sánchez et al., 2021). However, the use of these caused to search for alternatives having a high rate of drug resistance, toxicity risk, and difficulties of application. In addition, the observation of undesirable side effects was limited due to lack of activity and lack of pharmacokinetics. This necessitates the continual synthesis of new agents in scientific work in recent years. The main structure of most compounds that are used today as drugs, especially triazole, thiadiazoles, and oxadiazole, contains a five-membered heterocyclic ring structure. According to the arrangement of heteroatoms in the ring, there are various isomers of these rings. However, the most biologically active isomers among these isomers are as follows; 1,3,4-thiadiazole, 1,3,4-oxadiazole, and 1,2,4-triazole. The fact that triazole derivatives have potential application areas such as the ability to form multimolecular ligands, to be used as drug active ingredients, pesticides, artificial acceptor (Zhou and Wang, 2012; Sathish Kumar and Kavitha, 2013; Pereira et al., 2021) makes these substances a very attractive field of research. A triazole ring having three nitrogen atoms and a system enriched in terms of electrons is significant as a heterocyclic compound having five members. When we consider its characteristics, it has aromatic features as well. Derivatives of triazole has the ability to bond enzymes and receptors in the biological system. While performing such binding, it uses coordination linkage, ion-dipole force, cations- π , π - π , Van der Waals bonds, and hydrophobic effect. As a result, these compounds reveal various biological effects (Mi et al., 2008; Zhou and Wang, 2012; Küçükgül and Çıkla-Süzgün, 2015). Another characteristic of triazole compounds is the ability to form multimolecular structures (Rodríguez-Fernandez et al., 2005; Liu et al., 2011). Therefore, special attention is paid to triazole compounds, especially during new multimolecule drug development (Zhou et al., 2009; Zhou et al., 2010; Castro et al., 2012; Parchenko et al., 2012).

Moreover, triazole ring could also be preferred as a tool for binding in forming bi-functional drug molecules and for combining different pharmacophore moieties. Thus, it provides a usable and efficacy way to improve different biologically active and practical compounds.

Triazoles are used in the pharmaceutical industry, as an anti-tuberculosis, in the paint industry, production of agricultural chemicals, obtaining photographic materials, and anti-corrosion materials, but one of the most important areas of their use is undoubtedly the area of chemotherapy. These compounds containing five-membered rings have a very comprehensive active biological spectrum and are particularly useful in anticancer activity, also as an antifungal, anti-HIV, antitumor, antibacterial, antiviral, antidepressant, anti-inflammatory, anti-tuberculosis, analgesic, diuretic substances

(Tozkoparan et al., 2000; Turan-Zitouni et al., 2001; Cottineau et al., 2002; Olesen et al., 2003; Mocharla et al., 2005; Shiradkar et al., 2007; Sztanke et al., 2008; Bakunov et al., 2010; Khan et al., 2010; Menendez et al., 2011; Patpi et al., 2012; Khan et al., 2014).

Cyclobutane derivatives are widely used (Omer et al., 2022). For example, phenyl cyclobutane carboxylic acid is one of the esters of estrogenic prostaglandins. According to the literature, a study (Roger et al., 1977) has found that compounds of naphthalene and its derivatives containing cyclobutane are biologically active compounds in the structure. These compounds have been used against neurological diseases. It has also started to be used instead of widely used reserpine medicine. It is fact that cyclobutane carboxylic acid derivatives shows the sedative characteristics of antidepressants (Roger et al., 1977). Also, cyclobutane derivatives are known to have anti-inflammatory and anti-depressant activities (Dehmlow and Schmidt, 1990).

It has been noted that pyridine compounds are useful as being anti-cancer (Abbas et al., 2015; Mosaad et al., 2012) anti-microbial (Butnariu et al., 2007; Gaonkar et al., 2007; Kandile et al., 2009; Bhardwaj et al., 2016) and anti-fungal (Rajput and Sharma, 2011), anti-inflammatory and analgesic (Dogruer et al., 2007; Hosni and Abdulla, 2008; Gökçe et al., 2009), anti-hiv (Ali et al., 2007), anti-plasmodial (Kumar et al., 2008), anti-tubercular (Lourenco et al., 2007), antibacterial (Sharma and Jain, 2008), anticonvulsant (Rubat et al., 1990; Rastkari and Sharifzadeh, 2004), COX inhibitor (Chintakunta et al., 2002), antidiabetic (Rathish et al., 2009), antihypertensive (Barbaro et al., 2001; Joule et al., 2020), anti-cancer (Malinka et al., 2004; Brana et al., 2005; Atanasova et al., 2007; Karki et al., 2010), platelet aggregation inhibitors, anxiolytic (Griebel et al., 1999; Sotelo et al., 2002), antioxidant (Wermuth et al., 1989; Chen et al., 1997; Zhang et al., 1999; Çalişkan-Ergün et al., 2008), antiviral and antitumor (Blanco et al., 1999; Graf-Christophe et al., 2000). To illustrate, isoniazid and amlodipine contain pyridine in terms of being anti-tuberculosis and anti-hypertensive. Furthermore, antiviral, antineoplastic, antiproliferative effects of derivatives of the cyclobutane ring with other biologically active compounds and the contribution of the cyclobutane group to the enrichment of these properties are being tried to be explained (Blanco et al., 1999; Graf-Christophe et al., 2000). Based on the above findings, the pyridine structure is regarded as a distinctive structure and consequently attracted the general and continuous interest of synthetic organic chemists.

Because of these biological and pharmacological effects, it is envisaged that compounds containing cyclobutane, triazole, and pyridine in the structure will be used especially in the pharmaceutical industry. For this purpose, pyridine based 1,2,4-triazole compounds, which are not found in the literature, have been synthesized and investigated with biological potential.

Recently, cancer has become one of the most alerting diseases for human beings. and the prevalence of cancer has been

increasing and definite treatment for cancer has not been found yet. New cancer drugs are being developed with billions of dollars spent on cancer treatment, but some of them are out of use due to serious side effects and durability. For this reason, new drug compounds with high therapeutic efficacy and low side effects are still under study for molecular synthesis and have become a focus of interest for scientists. The prevalence of colon cancer is seen highly among gastrointestinal diseases. Nearly, 10% of the cases of cancer seen in males and females is to be colorectal cancer (Jemal et al., 2009). These cancers are also caused by mitochondrial mutations as well as genomic mutations (Aral and Özer, 2007; Gürbüz et al., 2011). Chemotherapy is a very important and basic treatment for colon cancer (Segal and Saltz, 2009).

In recent years, research in chemistry and biochemistry has been accelerated to explain behaviors (platinum group metal complexes) as cytotoxic markers, speeding up the search for platinum and various transition metals as new antitumor markers, and continuing to study new metal complexes that are not cisplatin but have properties of antitumor compounds. Cisplatin is used in the treatment of neoplastic diseases and its effect depends on its interaction with Dna (Stubbert et al., 2010). Cis-platinum, carboplatin, oxaplatin, nedaplatin, and lobaplatin compounds are known as the main compounds of important compounds. However, clinical applications have shown serious side effects of cisplatin anti-carcinogens (Khan et al., 2011). For this purpose, new searches are ongoing. New quests are underway for this purpose. Alternative searches continue because side effects of compounds used in cancer therapy limit their practice.

Various studies regarding the anticancer effect of 1,2,4-triazole ringed derivatives in the literature are inconsistent with the results of our study. It was emphasized in the study that compounds having 1,2,4-triazole pyridine rings are potent anti-cancer agents due to in vitro activities (Wang et al., 2013, Qin et al., 2014). It has been noted that 2-(4-(2-(dimethylamino) ethyl)-4H-1,2,4-triazole-3-pyridine derivatives were revealed in-vitro cytotoxic activity for five different types of cancer cells including MKN-45, H460, HT-29, A549, and U87MG in addition to normal ones (Qin et al., 2014). In another study, triazole derivative compounds were reported to be potent anti-cancer as the result of using the method of MTT. This technique has been frequently preferred as a test for enzymes in terms of the evaluation of cytotoxicity, in which cytotoxic effects were examined in HCT-116, U-87-MG, and MCF-7 cancer cells. The researchers have noted compounds having 1,2,4-triazole pyridine rings were considered antiproliferative as a result of in vitro activity and that they were potent anti-cancer agents (Wang et al., 2013). In a study, it was determined that HT29 cells were more than 75% cell viability after 3 days when 4 μM (12 $\mu\text{g}/\text{ml}$) dose of cisplatin was administered (de Mattos et al., 2008). In another study conducted by Suzuki et al., it has been reported the IC50 value when cisplatin was administered to DLD1 cells, the colorectal cancer cell line, was 78.4 μM (Suzuki et al., 2010). Another study found that when cisplatin was administered to HT29 cells for 72 h, the dose of IC50 corresponding to the dose required to kill half of the cells in 75 μM concentration was reached (Gürbüz et al., 2011).

The heterocyclic compounds such as cyclobutane derivatives, 1,2,4-triazole, and pyridine rings have shown satisfactory antioxidant and cytotoxicity. It seems that the hybridization of

these skeletons would also create more effective agents. In this work, we focused on the design, synthesis, and evaluation of new cyclobutane containing triazoles derivatives (5a-e) as anti-cancer, antimicrobial, and antioxidant agents.

The purpose of the study is to perform the synthesis of five new compounds by using methods found in academic reviews as cyclobutane derivative 1,2,4-triazole series (5a-e) with the pyridine ring. The process of synthesis was performed on the compounds and featured experimentally via IR, ^1H NMR, ^{13}C NMR methods. In addition, antioxidant, antimicrobial, and anticancer properties of synthesized compounds were examined.

2. Materials and methods

2.1. Chemicals properties

The hydrogen nuclear magnetic resonance (^1H NMR) and 13-carbonnuclear (^{13}C NMR) were performed on Bruker ascend 400 nuclear magnetic resonance spectrometers at the speed of 400 MHz for ^1H and the speed of 100 MHz for ^{13}C . Deuterated dimethyl sulfoxide (DMSO d_6) was used for dissolving the NMR compound. The examination in terms of elemental was carried out on a LECO-CHNS-938. The chemicals used were supplied from Merck. Thiosemicarbazides compounds (2a-e), 4-(aryl-alkyl)-5-(2-furanyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (3a-e) and 2-chloro-1-(3-Methyl-3-mesityl-cyclobutyl)-ethanone (4) were synthesized according to the general procedure described earlier (Koparir et al., 2011; Koparir, 2019). These five test compounds (5a-e) are given in Table 1 and Supplemental File (IR, ^1H NMR and ^{13}C NMR). The synthesis and mechanism of test compounds are given in Scheme 1.

General synthesis of 1-(3-Methyl-3-mesityl)-cyclobutyl-2-(5-pyridine-2yl-4-(aryl-alkyl)-4H-[1,2,4]triazol-3-ylsulfanyl)-ethanone (5a-e): A solution of 0.445 g (2 mmol) of 2-chloro-1-(3-Methyl-3-mesityl-cyclobutyl)-ethanone (4) was dissolved in dry acetone (30 mL) containing (2 mmol) K_2CO_3 . To this solution, (2 mmol) of 4-(aryl-alkyl)-5-(2-pyridine)-2,4-dihydro-3H-1,2,4-triazole-3-thione (3a-e) in dry acetone (20 mL) was added dropwise over a 1 h period at room temperature with stirring. The resulting solid was collected by filtration, dried and recrystallized from ethyl alcohol.

2.2. Biological properties

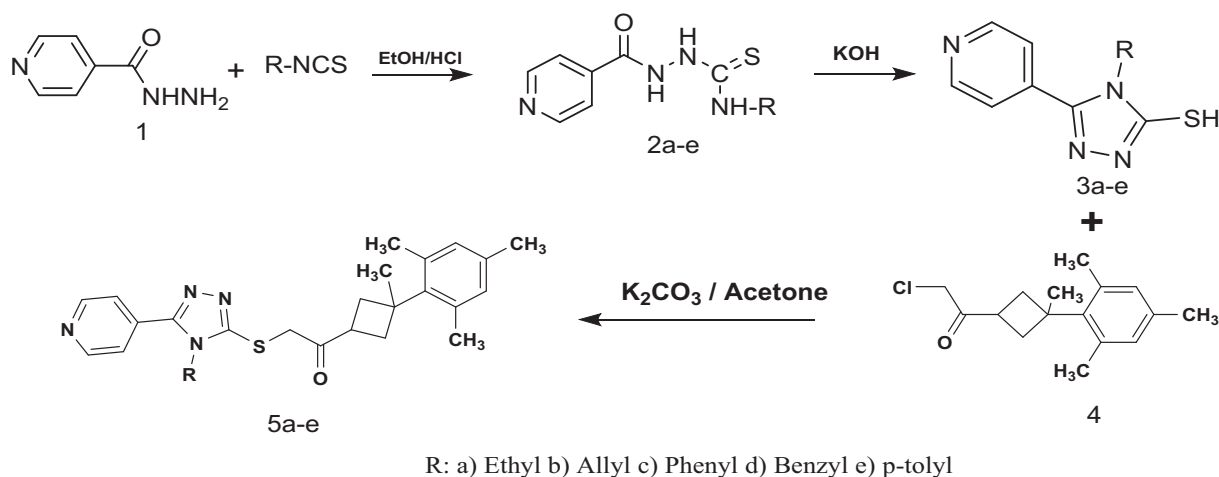
2.2.1. Anticancer activity

The human colon adenocarcinoma cell line HT29 was used to determine anticancer activity. The frozen HT29 human colon adenocarcinoma cells which were taken from the bank of cell culture (Sigma, USA) have been thawed at room temperature. It was then taken to a flask of 75 mL. DC5 (25 mL) was already in the flask. Then the flask was put in a 5% CO_2 incubator a Nuaire brand (Plymouth, MN, USA). The condition of cells was examined by means of a microscope SoFi brand (Soif Optical Inc., China) daily. A new DC5 was put instead of the previous one on the third day. This procedure was carried out for three days by specific intervals.

The number of cells has increased moving on top of each other and then they covered the surface of the flask. On the 15th day, the medium was removed, and a new 3 mL of trypsin was added and put into the incubator. Shaking was performed

Table 1 Physical and analytical data of test compound (5a-e).

Code of Compounds	Nomenclature of compounds	Description
5a	1-(3-Methyl-3-mesityl)-cyclobutyl-2-((5-(pyridine-4-yl)-4-ethyl-4H-[1,2,4]triazol-3-yl)sulfanyl)-ethanone	Yield 67%; m. p. 150–152 °C; FT-IR (KBr, cm^{-1} , ν): 2945–2982 (Ar-H), 2870–2930 (C-H), 1701 (C = O), 1432 (C = C), 1386 (C = N), 1125 (C-N), 1042 (N-N), 713 (C-S); ^1H 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 (mesityl)), 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 (mesityl)), 7.68 (d, 2H, Ar-H, J = 4.5 Hz, C = CH aromatic (pyridine)), 8.76 (d, 2H, Ar-H, J = 4.4 Hz, N = CH aromatic (pyridine)). ^{13}C NMR (100 MHz, DMSO d_6 , δ , ppm): 15.4, 20.5, 21.4, 25.2, 39.1, 40.3, 41.2, 122.7, 130.5, 134.4, 143.7, 150.6, 151.7, 153.1, 205.3. Calcd. for 434.59 (C ₂₅ H ₃₀ N ₄ OS). (%):C, 69.09; H, 6.96; N, 12.89; S, 7.38; found (%): C, 69.01; H, 6.90; N, 12.85; S, 7.45.
5b	1-(3-Methyl-3-mesityl)-cyclobutyl-2-((5-(pyridine-4-yl)-4-allyl-4H-[1,2,4]triazol-3-yl)sulfanyl)-ethanone	Yield 72%; m. p. 115–117 °C; FT-IR (KBr, cm^{-1} , ν): 2950–2990 (Ar-H), 2862–2914 (C-H), 1710 (C = O), 1435 (C = C), 1395 (C = N), 1126 (C-N), 1013 (N-N), 705 (C-S); ^1H NMR (400 MHz, DMSO d_6 , δ , ppm): 1.49 (s, 3H, -CH ₃ (cyclobutane)), 2.14 (s, 9H, -CH ₃ (mesityl)), 2.39–2.50 (m, 4H, -CH ₂ - (cyclobutane)), 3.57 (p, 1H, -CH- (cyclobutane), J = 8.70 Hz), 4.33 (s, 2H, S-CH ₂ -), 4.74 (m, 2H, -N-CH ₂ -CH-CH ₂), 4.86 (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} = 9.8 Hz), 5.96–6.02 (m, 1H, N-CH ₂ -CH-CH ₂), 6.70 (s, 2H, Ar-H (mesityl)), 7.64 (d, 2H, Ar-H, J = 4.2 Hz, C = CH aromatic (pyridine)), 8.75 (d, 2H, Ar-H, J = 4.4 Hz, N = CH aromatic (pyridine)). ^{13}C NMR (100 MHz, DMSO d_6 , δ , ppm): 20.5, 21.4, 25.2, 39.1, 41.3, 47.1, 117.7, 122.4, 130.4, 132.6, 134.4, 134.9, 143.7, 150.9, 152.5, 153.6, 205.3. Calcd. for 446.6 (C ₂₆ H ₃₀ N ₄ OS). (%):C, 69.92; H, 6.77; N, 12.54; S, 7.18 (%): C, 69.85; H, 6.72; N, 12.50; S, 7.25.
5c	1-(3-Methyl-3-mesityl)-cyclobutyl-2-((5-(pyridine-4-yl)-4-phenyl-4H-[1,2,4]triazol-3-yl)sulfanyl)-ethanone	Yield 66%; m. p. 145–147 °C; FT-IR (KBr, cm^{-1} , ν): 2941–3046 (Ar-H), 2863–2937 (C-H), 1709 (C = O), 1432 (C = C), 1334 (C = N), 1125 (C-N), 1035 (N-N), 699 (C-S); ^1H NMR (400 MHz, DMSO d_6 , δ , ppm): 1.50 (s, 3H, -CH ₃ (cyclobutane)), 2.14 (s, 9H, -CH ₃ (mesityl)), 2.40–2.55 (m, 4H, -CH ₂ - (cyclobutane)), 3.57 (p, 1H, -CH- (cyclobutane), J = 8.60 Hz), 4.30 (s, 2H, S-CH ₂ -), 6.71 (s, 2H, Ar-H (mesityl)), 7.29 (d, 2H, Ar-H, J = 4.4 Hz, C = CH aromatic (pyridine)) 7.46–7.48 (m, 2H, Ar-H), 7.59–7.63 (m, 3H, Ar-H), 8.55 (d, 2H, Ar-H, J = 4.4 Hz, N = CH aromatic (pyridine)). ^{13}C NMR (100 MHz, DMSO d_6 , δ , ppm): 20.5, 21.4, 25.2, 39.0, 40.8, 121.9, 127.9, 130.5, 131.0, 133.8, 134.2, 134.4, 134.9, 143.7, 150.6, 152.6, 153.4, 205.0. Calcd. for 482.6 (C ₂₉ H ₃₀ N ₄ OS). (%):C, 72.17; H, 6.27; N, 11.61; S, 6.64 (%): C, 72.25; H, 6.32; N, 11.55; S, 6.57.
5d	1-(3-Methyl-3-mesityl)-cyclobutyl-2-((5-(pyridine-4-yl)-4-benzyl-4H-[1,2,4] triazol-3-yl)sulfanyl)-ethanone	Yield 79%; m. p. 120.9 °C; FT-IR (KBr, cm^{-1} , ν): 2969–3042 (Ar-H), 2854–2945 (C-H), 1716 (C = O), 1423 (C = C), 1363 (C = N), 1117 (C-N), 1036 (N-N), 698 (C-S); ^1H NMR (400 MHz, DMSO d_6 , δ , ppm): 1.49 (s, 3H, -CH ₃ (cyclobutane)), 2.14 (s, 9H, -CH ₃ (mesityl)), 2.48–2.55 (m, 4H, -CH ₂ - (cyclobutane)), 3.55 (p, 1H, -CH- (cyclobutane), J = 8.80 Hz), 4.32 (s, 2H, S-CH ₂ -), 5.38 (s, 2H, N-CH ₂ -C ₆ H ₅), 6.70 (s, 2H, Ar-H (mesityl)), 7.00–7.02 (m, 2H, Ar-H), 7.25–7.34 (m, 3H, Ar-H), 7.57 (d, 2H, Ar-H, J = 4.8 Hz, C = CH aromatic (pyridine)), 8.68 (d, 2H, Ar-H, J = 4.8 Hz, N = CH aromatic (pyridine)). ^{13}C NMR (100 MHz, DMSO d_6 , δ , ppm): 20.5, 21.4, 25.2, 39.0, 41.2, 48.1, 122.5, 126.7, 128.4, 129.4, 130.5, 134.4, 134.7, 134.9, 135.5, 143.7, 150.9, 152.8, 153.7, 205.3. Calcd. for 496.7 (C ₃₀ H ₃₂ N ₄ OS). (%):C, 72.55; H, 6.49; N, 11.28; S, 6.46 (%): C, 72.40; H, 6.55; N, 11.35; S, 6.55.
5e	1-(3-Methyl-3-mesityl)-cyclobutyl-2-((5-(pyridine-4-yl)-4-(<i>p</i> -tolyl)-4H-[1,2,4]triazol-3-yl)sulfanyl)-ethanone	Yield 71%; m. p. 145–147 °C; FT-IR (KBr, cm^{-1} , ν): 2947–3043 (Ar-H), 2865–2918 (C-H), 1694 (C = O), 1428 (C = C), 1333 (C = N), 1121 (C-N), 1035 (N-N), 694 (C-S); ^1H NMR (400 MHz, DMSO d_6 , δ , ppm): 1.51 (s, 3H, -CH ₃ (cyclobutane)), 2.15 (s, 9H, -CH ₃ (mesityl)), 2.40 (s, 3H, C ₆ H ₅ -CH ₃), 2.42–2.56 (m, 4H, -CH ₂ - (cyclobutane)), 3.57 (p, 1H, -CH- (cyclobutane), J = 8.70 Hz), 4.28 (s, 2H, S-CH ₂ -), 6.71 (s, 2H, Ar-H (mesityl)), 7.31 (d, 2H, Ar-H, J = 4.7 Hz, C = CH aromatic (pyridine)), 7.32–7.40 (m, 4H, Ar-H), 8.57 (d, 2H, Ar-H, J = 4.5 Hz, N = CH aromatic (pyridine)). ^{13}C NMR (100 MHz, DMSO d_6 , δ , ppm): 20.5, 21.3, 21.4, 25.2, 39.0, 40.0, 40.8, 121.9, 127.7, 130.5, 131.2, 134.3, 134.4, 134.9, 140.8, 143.7, 150.6, 152.6, 153.4, 205.1. Calcd. for 496.6 (C ₃₀ H ₃₂ N ₄ OS). (%):C, 72.55; H, 6.49; N, 11.28; S, 6.46 (%): C, 72.40; H, 6.55; N, 11.40; S, 6.39.



Scheme 1 The synthesis scheme of test compounds (5a-e).

for the flasks nearly for 2–3 min in order to make cells separated from the basis attached. DC5 of 12 mL was added to the flask when all the cells were removed from the surface. The cells were distributed homogeneously into the solution by carefully triturating (the separation process by pulling the suspension into the pipette and emptying it).

Hemocytometer was used for cell counting. In each flask, a suspension of 1×10^5 was placed. Then, DC5 was mixed with them. When the volume reached 25 mL, the flasks were moved to the incubator. The cultivating, nutrition and experiments of the cells were carried out in Class II Laminar flow (Biolaf, Ankara) (Offiong and Martelli, 1997; Ferrari et al., 1999).

Centrifugation for HT29 human colon adenocarcinoma cell suspension was performed for 5 min at 2000 rpm. Hemocytometer was used for cell counting. The number of cells was identified as 1×10^5 / ml for the HT29 human colon adenocarcinoma cell experiments. Pre-trials were performed to determine the doses and the up-and-down technique was preferred. One ml was removed from the cell suspension and the agents were mixed at concentrations of 15, 30, 60 μ M. Serum having the same amount was mixed in negative control tubes. DMSO was also added to the physiological vehicle tubes. They were exposed to incubation. DMSO was about 1% in cell suspensions. 24 h later, incubation was stopped and pulverized. 100 cells were taken in a random manner in 1:1 (v/v) and then counted with 0.4% trypan blue in hemacytometer. The percentage was used as showing the value of cell viability. The same procedure applied after 48 h, and the test has been terminated (Kumamoto et al., 1990).

2.2.2. DPPH radical scavenging activity

There was not a major change found in the antioxidant activity of the compounds by using Blois method including 1,1-Diphenyl 2-picrylhydrazyl (DPPH) (Blois, 1958). This technique majorly focuses on free colorful radical 1,1-Diphenyl 2-picrylhydrazyl radical (DPPH) eliminations via free radical scavengers. DPPH has a red color, and it is a free radical. When free radicals are scavenged by antioxidant compounds, the color turns from red to yellow. There was a reduction in terms of absorption mixture at 517 nm. This has indicated

an increase in antioxidant activity in the free radical scavenging. Reagents used are: 0.1 mM DPPH; Butyl Hydroxy toluene (BHT) (1 mg/ml).

The compounds synthesized in the work were dissolved in ethyl alcohol as 1 mg/ml. The standard was dissolved in ethanol, again at 1 mg/ml. Test tubes were used to put test compound and standards in the amount of 50, 100, 250 μ g/ml respectively. In order to form a total volume of 3 mL, DMSO was used for the completion. 1 mL of the stock DPPH solution was put into the experimental tubes. The incubation process was initiated at room temperature and the room was kept dark. The duration was about 30 min. After incubation absorbance of (UV-Vis Spectrophotometer: Shimadzu UV-1700 Spectrophotometer), the ethanol-containing mixture was found to be 517 nm. Ethanol ve DPPH solutions were used in the amount of 3 mL, and 1 mL respectively. The decreased absorbance has revealed the scavenging activity of free radicals for the remaining 1,1-Diphenyl 2-picrylhydrazyl radical (DPPH) solution. The calculation for DPPH radical scavenging activity in the reaction medium was performed in accordance with the formulation presented below.

% DPPH Free Radical Scavenging Activity

$$= (A_0 - A_1 / A_0) \times 100.$$

A₀: Absorbance of the control reaction.

A₁: Absorption of the sample or standard.

2.2.3. Determination of minimum inhibitory concentrations (MICs) of antimicrobial agents

After the chemicals were synthesized, the anti-microbial activities of these chemicals were examined. The method of microdilution broth was preferred with a slight change (Eloff, 1998), two gr-positive bacteria *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 2921), two gr-negative bacteria *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) and single fungal strain *Candida albicans* (ATCC 10231) were used in order to determine this activity. Each chemical agent was dissolved in DMSO (5000 μ M) and then the dilution process was performed as 1/10 in sterile distilled water. Distilled water was

poured into the wells consisting of 96-well microtiter plates. The amount of the water was 50 μ l. The following step was pouring 50 μ l of the chemical compounds into the wells. Then two-fold dilutions were performed serially in a 96-well plate. The concentration of chemical compounds in wells ranged from 125 μ M to 0.48 μ M. MHB (Mueller Hinton Broth, Accu-mix® AM1072) and SDB (Saboraud Dekstro Broth, Himedia ME033) were preferred for diluting bacteria and *Candida* cultures. The size of the final inoculum was found to be 5×10^5 CFU/mL at bacteria and $0.5\text{--}2.5 \times 10^3$ CFU/mL at *Candida*. For the positive and negative controls, gentamicin and fluconazole were used respectively. The incubation was performed for microtiter plates at the temperature of 37 °C for bacteria and at the temperature of 35 °C for *Candida* for about 16–24 h. Then, the wells were filled with 50 μ l 2 mg/ml 2,3,5-Triphenyl tetrazolium chloride (TTC) (Meck, Germany) in terms of microbial growth. Then they were once again exposed to incubation at 37 °C for 2 h. A decrease was observed in the red color of formazan after the incubation process. This was considered to be an acceptable MIC value (Fig. 1). Findings obtained as a result of the study are given in Table 3.

2.2.4. Statistical analysis

Mean \pm standard error was used for presenting the data in the study. The analysis of the data was conducted by using One-way ANOVA and Tukey test for the anti-tumor. SPSS/PC package program was used for the analysis of the study.

3. Results and discussion

The 3,4,5-trisubstituted 1,2,4-triazoles obtained in the first step in this work are reacted with 2-chloro-1-(3-methyl-3-mesityl-cyclobutyl) ethanone in dry acetone containing K_2CO_3 to obtain the 4,5-trisubstituted 1,2,4-triazole sulfanyl compounds. 3,4,5-trisubstituted 1,2,4-triazoles reacted with 2-chloro-1-(3-methyl-3-mesityl-cyclobutyl)-ethanone in the presence of K_2CO_3 via the SN^2 reaction mechanism to yield the sulfanyl compounds containing the triazole and cyclobutane ring (Scheme 1). Efficient yields range from 66% to 79%. When the IR spectra of 5a–e in the 3,4,5-tri substituted 1,2,4-triazole sulfanyl compounds are examined, the most characteristic peak is the C=O peak. The C=O stretching vibration occurs in the range of 1694–1710 cm^{-1} . The C-S-C strain peculiar to the structure is around 694–716 cm^{-1} . Around 2941–3046 cm^{-1} Ar-H, around 2860–2920 cm^{-1} C-H, around 1160 cm^{-1} C-C peaks for the cyclobutane ring and C=C bands for the mesityl ring at 1600 cm^{-1} were obtained. When the proton NMR spectrum is examined, one of the characteristic peaks in the 3,4,5-trisubstituted 1,2,4-triazole sulfanyl is signalled by nine $-CH_3$ protons in the mesityl group. This signal appeared as a singlet at approximately 2.14 ppm and was found to be equivalent to nine hydrogen signals. The signal of $-CH_3$ in the cyclobutane ring appeared as a singlet at about 1.50 ppm. Four hydrogens in the cyclobutane ring were distinctly resonant as -cis and -trans. These four protons occurred as a multiplet at 2.45–2.55 ppm. Data for all compounds are given in Table 1.

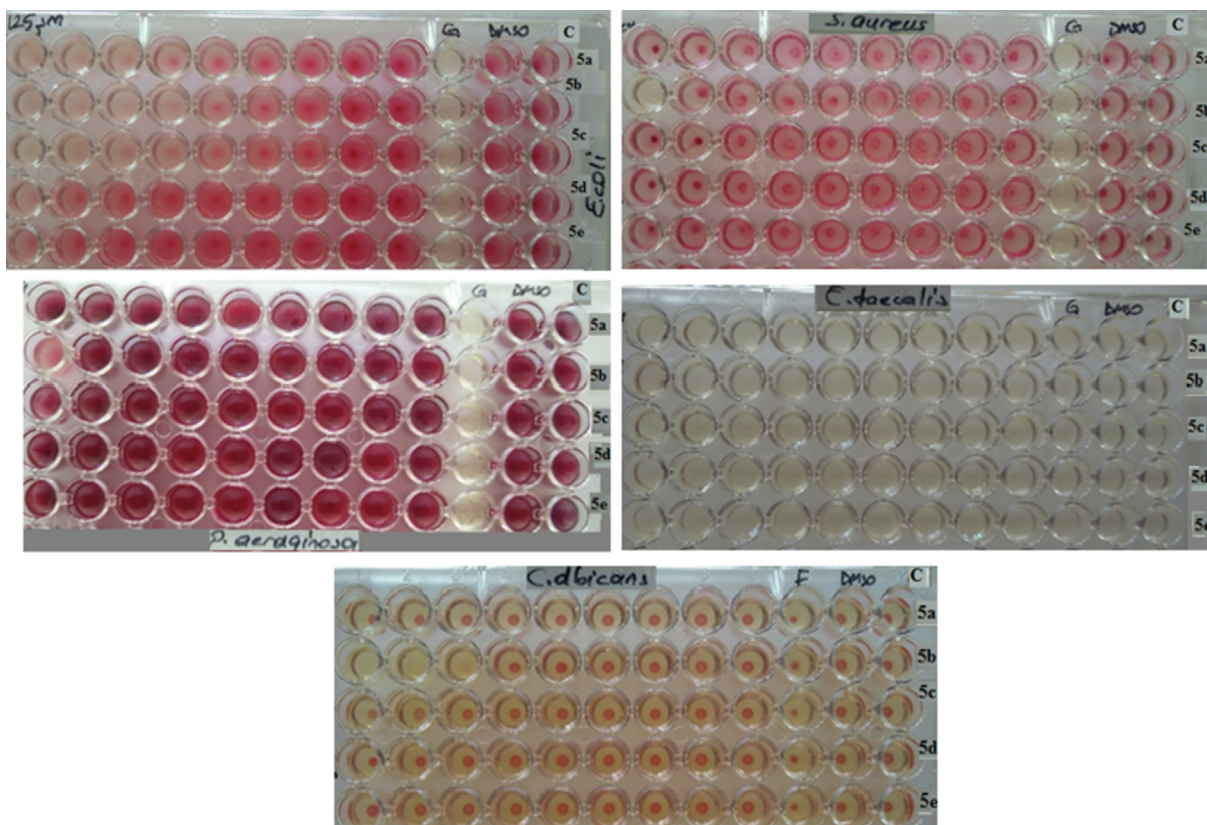


Fig. 1 Determination of minimum inhibitory concentrations (MICs) of antimicrobial agents for compounds (5a-e).

In this study, we applied five compounds encoded as 5a-e to ht 29 human colon adenocarcinoma cells, findings of our compounds' anticancer properties have been shown in Table 1. according to the results, it was found that all of our test substances destroyed the cancerous cells in a statistically significant manner when we compared them with the control group. compounds were observed to kill ht 29 human colon adenocarcinoma cells, in a statistically significant ($p < 0.05$) way when we compared with the control group. the study was conducted on three different concentrations (15 μM , 30 μM , 60 μM).

When Table 2 and Fig. 1 of 5a-e coded test compounds were examined, it was determined that there were different statistical results between the groups at these concentrations ($p < 0.05$). According to the results obtained, it can be said that the higher concentrations of triazole compounds we use are more effective in destroying cancerous cells. Living cell results of HT 29 human colon adenocarcinoma cells exposed to substances were presented in Table 2 and Fig. 2.

Fig. 2, was also showing the results accordingly. It was noted that the difference among the groups having different substituents on triazole ring on the same line was statistically significant.

For the antimicrobial study, bacteria *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. coli* and a single fungal strain *C. albicans* were used. The results were shown in Table 3. As shown in Table 2, antimicrobial activities had different variations. MIC values of the chemicals were between 15.625 - > 125 μM . The 5b was found the most effective chemical which it was effective on *E. coli*, *S. aureus*, *P. aeruginosa*, *E. faecalis* and *C. albicans*. *E. coli* was noted to be more sensitive compared to *S. aureus*, *P. aeruginosa*, *E. faecalis*, and *C. albicans* against the 5a and 5c chemicals. MIC values for the synthesized compounds were found as > 125 μM except for 5b, 5a, and 5c.

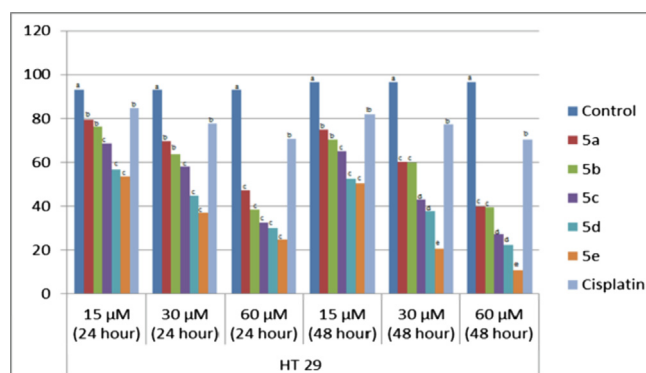


Fig. 2 Cell viability, %, rates relative to doses and time in HT29 cell lines after treatment with triazole compounds (5a-e).

In vitro DPPH free radical scavenging activities were shown in Table 4. It has been noted that 5c and 5e of the compounds showed activity close to the standard antioxidant BHT. On the

Table 4 DPPH radical scavenging (%) activity of synthesized compounds (5a-e).

Groups	50 $\mu\text{g/mL}$	100 $\mu\text{g/mL}$	250 $\mu\text{g/mL}$
5a	18.90 \pm 2.12 ^a	29.33 \pm 1.02 ^c	25.18 \pm 2.02 ^c
5b	20.05 \pm 0.14 ^a	22.16 \pm 1.59 ^{ab}	24.66 \pm 0.46 ^{bc}
5c	24.48 \pm 0.88 ^b	48.20 \pm 0.19 ^d	46.75 \pm 1.67 ^d
5d	22.48 \pm 2.02 ^{bc}	39.33 \pm 1.02 ^{cd}	38.44 \pm 2.50 ^c
5e	26.50 \pm 1.11 ^c	42.33 \pm 0.22 ^d	48.50 \pm 0.19 ^d
BHT	30.2 \pm 0.22 ^c	45.2 \pm 0.99 ^d	50.3 \pm 0.38 ^d

Data are presented as mean \pm SD a-d; the difference between groups with different letters on the same line is statistically significant at $P < 0.05$.

Table 2 Interaction of triazole substances (5a-e) and % cell viability rates on human colon carcinoma (HT29) cells lines.

Groups	24 h		48 h		48 h	
	15 μM	30 μM	60 μM	15 μM	30 μM	60 μM
Control	93.14 \pm 0.78 ^a	93.14 \pm 0.78 ^a	93.14 \pm 0.78 ^a	96.64 \pm 0.16 ^a	96.64 \pm 0.16 ^a	96.64 \pm 0.16 ^a
5a	79.33 \pm 1.80 ^b	69.54 \pm 1.60 ^b	47.40 \pm 1.96 ^c	74.75 \pm 0.85 ^b	60.25 \pm 0.48 ^c	39.75 \pm 0.18 ^c
5b	76.15 \pm 1.75 ^b	63.60 \pm 2.04 ^b	38.33 \pm 1.44 ^c	70.25 \pm 1.44 ^b	59.75 \pm 1.25 ^c	39.50 \pm 0.65 ^c
5c	68.67 \pm 1.03 ^c	57.93 \pm 0.91 ^c	32.42 \pm 0.63 ^c	65.25 \pm 1.03 ^c	43.00 \pm 0.41 ^d	27.25 \pm 1.11 ^d
5d	56.75 \pm 1.89 ^c	44.82 \pm 1.19 ^c	30.02 \pm 0.41 ^c	52.50 \pm 1.32 ^c	37.75 \pm 1.10 ^d	22.25 \pm 0.85 ^d
5e	53.52 \pm 1.55 ^c	37.03 \pm 1.49 ^c	24.85 \pm 1.08 ^c	50.25 \pm 1.49 ^c	20.75 \pm 0.85 ^e	10.75 \pm 0.35 ^e
Cisplatin	84.70 \pm 1.06 ^b	77.70 \pm 0.37 ^b	70.80 \pm 0.26 ^b	81.90 \pm 1.12 ^b	77.50 \pm 0.33 ^b	70.42 \pm 0.9 ^b

^{a-d} The difference between the groups with different letters on the same line is statistically significant ($P < 0.05$).

Table 3 Antimicrobial activity of test compounds (μM minimum inhibition concentration values).

Groups	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. faecalis</i>	<i>C. albicans</i>
5a	15.625	> 125	> 125	> 125	> 125
5b	15.625	125	125	> 125	31.25
5c	15.625	> 125	> 125	> 125	> 125
5d	> 125	> 125	> 125	> 125	> 125
5e	> 125	> 125	> 125	> 125	> 125
Fluconazole	> 125	> 125	> 125	> 125	> 125
Gentamicin	> 125	> 125	> 125	> 125	> 125

other hand, 5d test compounds have been found to have a moderate activity, and 5a and 5b test compounds have a low activity (Fig. 3).

In this study, it was demonstrated that after 15 μM cisplatin administration, it is above 80% of the viability of HT29 cells (Fig. 2).

These results suggest that colon cancer cell lines and indirectly cancerous tissues may be resistant to cisplatin at a certain concentration. It is therefore thought to be useful to overcome the cisplatin resistance observed in the treatment of colon cancer with newly synthesized compounds and provide alternative treatment options. The anticancer activities of the test compounds in the 24- and 48-hour period are shown as $5e > 5d > 5c > 5b > 5a$ respectively. Test compounds were found to be highly effective in 48 h and high doses compared to the control group and cisplatin. It should be investigated whether there are different side effects of these compounds which are more effective than cisplatin. It is also planned to investigate the toxicity of these compounds in the future.

When we look at the substituents on compounds that exhibit good antimicrobial activity from the test compounds, the presence of allyl-, phenyl- and ethyl cyclobutane substituents is evident. Although there are very similar structures on all compounds, it is interesting that they exhibit very different antimicrobial activities. Perhaps the electron-donating property of these compounds can be a reason for their antimicrobial activity. Generally, similar materials in terms of structure have been synthesized and antimicrobial effects are observed at different degrees (Ahmedzade et al., 2003). The skeletons of the compounds are the same, but their substituents are different. Therefore, the idea for the cause is the emergence of antimicrobial activity values at different levels. So, there should be more exhaustive studies to learn the plausible mechanisms related to the inhibition of the growth of antimicrobial organisms.

According to the results of antioxidant results, it has been noted that 5c and 5e of the compounds showed activity close

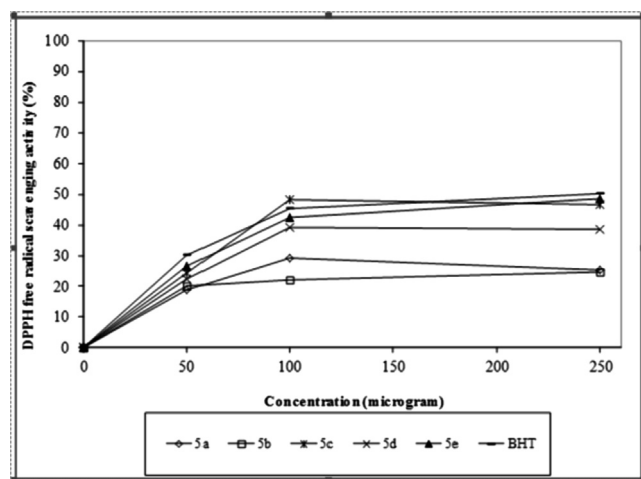


Fig. 3 Graphical representation of % DPPH free radical scavenging activity of synthesis compounds and standard antioxidant compound BHT and at the concentrations (50–100–250 $\mu\text{g}/\text{mL}$) for (5a-e).

to the standard antioxidant BHT. On the other hand, 5d test compounds have been found to have a moderate activity and 5a and 5b test compounds have low activity. Probably these results can show that our test compounds 5c and 5e have good activity as hydrogen donor because antioxidants on radical scavenging effects is considered to be originated from its hydrogen donor capabilities. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) is accepted to be a stable free radical. It becomes a stable molecule when an electron or hydrogen radical is accepted (Baumann, 1979; Soare et al., 1997).

4. Conclusion

In conclusion, when the results obtained from this study are evaluated, it has been found some of the compounds which were exposed to synthesis have anti-tumor and anti-bacterial effects. We believe that the results obtained in this study will lead to the synthesis of different substituted derivatives of this material and investigation of its different activities in future studies.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.arabjc.2022.103957>.

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