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Original Article

Synthesis, characterization and antimicrobial activity of some novel 4-amino-5- phenyl-4H-1,2,4-triazole-3-thiol derivatives

Nurhan Gumrukcuoglu[1], Muhammad Imran[2], Inam Iqbal[2]

[1]Karadeniz Technical University, Vocational School of Health Sciences, Trabzon, Turkiye
[2]Jubail Industrial College, General Studies Department, Al Jubail, Saudi Arabia

ABSTRACT

Background and aims: The discovery of new antifungals and antimicrobials to overcome resistance has always been a crucial topic for sustainable world health. Since sulfur-containing triazole heterocycles derivatives have shown greater interest due to their valuable applications, we reported herein, the synthesis of some mercaptotriazole derivatives to discover underlying structural requirements for antimicrobial and antifungal activity.

Methods: Firstly, the benzoic acid hydrazide was synthesized. Then it was reacted with carbon disulfide in the solution of alkali ethanol to give potassium dithiocarbazinate salt. Then the basic nucleus 4-amino-5-phenyl-1-4H-1,2,4-triazole-3-thiol was prepared by cyclization of potassium salt with hydrazine hydrate. After that, a condensation reaction with different aldehydes was conducted to synthesize Schiff bases, which were cyclized by treating with thioglycolic acid to prepare desired compounds.

Results: All the synthesized compounds were confirmed by their melting point, FTIR, 1H-NMR, and 13C-NMR spectra, elemental analysis was determined for their antimicrobial activity by using a simple susceptibility screening test with agar-well diffusion. Few compounds showed promising activity against bacteria and yeast-like fungi.

Conclusion: 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol derivatives proved promising antimicrobial activities.

Keywords: Triazole-3-thiol, antimicrobial activity, Schiff base, thiazolidenon derivative, triflucan

INTRODUCTION

Synthesis and development of new and safe therapeutic values containing chemical compounds, to avoid resistance as well as to increase selective effectiveness are taking the attention of worldwide researchers and scientists, particularly nitrogen-containing heterocyclic are mostly found in significant therapeutic agents. In this regard, the synthesis of some mercaptotriazole derivatives to discover underlying structural requirements for antimicrobial and antifungal activity was conducted.

The usage of most antimicrobial agents is now very limited, mainly because of rapidly developing drug resistance but also because of the unsatisfactory result of present bacterial and fungal infection treatments and side effects (Fidler, 1998). In the last few decades, great consideration has been dedicated to the synthesis of 1,2,4-triazole derivatives possessing such comprehensive bioactivities as antibacterial, antifungal (Karabasanagouda, Adhikari, & Shetty, 2006; Sztanke, Pasternak, Rzymowska, Sztanke, & Kandefer-Szerszeń, 2008), antimycobacterial (Klimesova, Zahajska, Waisser, Kaustova, & Mollmann, 2004), anti-inflammatory (Mullican et al., 1993), analgesic (Tozkoparan, Kupeli, Ozalp, & Ertan, 2005), anticancer (Demirbas, Ugurluoglu, & Demirbas, 2002) antihypertensive (Wright et al., 1986), anticonvulsant (Küçükgüzel et at., 2004), antiasthmatic (Youichiro et al., 1996), antiviral (El-Essawy, El-Sayed, El-Kafrawy, Morshedy, & Abdel-Rahman, 2008) diuretic (Shah, Mhasalkar, Patki, Deliwala, & Sheth, 1969), antidepressant (Kane, Dudley, Sorensen, & Miller, 1988) and hypoglycemic (Blank, Nichols, & Vaidya, 1972) activities. Although both, imidazole and triazole, are five-membered ring heterocycles, imidazole contains two ring nitrogen atoms, while triazoles have three. Nevertheless, when compared with imidazole (clotrimazole, ketoconazole, miconazole), triazoles are less susceptible to metabolic degradation and have much greater target specificity, increased potency, and an expanded spectrum of activity.

Sulfur containing triazole heterocycles are also very attractive to scientist because of their significant practical applications, particularly mercapto-and thione-substituted 1, 2, 4-triazoles are well known important compound (Sobhi et al.,

Corresponding Author: Muhammad Imran **E-mail:** imran_m@rcjy.edu.sa
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2022; Shcherbyna et al., 2022; Karpun & Polishchuk, 2021; Sameliuk, Zedan, & Kaplaushenko, 2021; Desai et al., 2021).

Triazole derivatives are also taking attention due to its valuable application in medicine (Kazeminejad et al., 2022; Zveaghintseva et al., 2021; Zazharskyi et al., 2021; Vagish, Sudeep, Jayadevappa, & Ajay Kumar, 2020; Benhammadi, Salimairaten, & Othman, 2016; Kumari et al., 2021; Mohamed, Sheha, Hassan, Abdel-Hafez, & Omar, 2018; Cavusoglu, Yurttas, & Canturk, 2018; Popiołek, Paruch, Patrejko, Biernasiuk, & Wujec, 2016; Sekhar et al., 2018; Xie et al., 2017; Wu et al., 2018), agriculture (Subhas, Sindhu, & Sreeveena, 2019; Shang et al., 2012; Yang, He, & Zhu, 2006; Howatt, 2005; Zhang, Damu, Cai, & Zhou, 2014) and, industry (Nazarov, Miroshnichenko, Ivakh, & Pyshyev, 2023; Yan, Jinchao, Yang, & Cheng, 2022; Popova et al., 2021; Shevtsov et al., 2020; Yin et al., 2009; Ueda & Nagasawa, 2009; Yeung & Farkas, 2005; Huntsman & Balsells, 2005; Zhou &Wang, 2012). Furthermore, some triazoles are recognized and used as analytical reagents (Seebunrueng, Tamuang, Ruangchai, Sansuk & Srijaranai, 2021; Liu et al., 2021; Wang, He, Chen, & Hu, 2020; Li, He, Chen, & Hu, 2019), dyes (Tkach et al., 2023; Diogo et al., 2023; Ma et al., 2023; Bakr, Abdel-Wahab, Bekheit, Mashaly, & Fahmy, 2023) and photographic chemicals (Ahmed et al., 2022; Koparir, Parlak, Karatepe, & Omar, 2022; Shimada, Ito, Maeta, Matsuoka, & Sato, 2006) and in the polymers preparation (Li et al., 2023; Sloop, 2023).

MATERIALS AND METHODS

General

The reagents used in the reactions were purchased commercially from Sigma Aldrich and Merck. Melting points were examined on the Barnstead Electro-thermal melting point device. ^{1}H-NMR and ^{13}C-NMR spectra (δ, ppm) were observed on a Varian Mercury 200 MHz spectrophotometer as a standard substance using tetramethyl silane.

Match constants (J values) were given as Hertz. NMR coefficients are truncated as follows: s= singlet, d= doublet, t= triplet, m= multiplet signal. The IR spectra (υ, cm^{-1}) were viewed with a Perkin-Elmer 1600 FTIR spectrometer in KBr pellets. Compounds **(1-5)** were synthesized benefiting a published method (Selvaraj et al., 2011, Čačić et al., 2010) (scheme-1). Elemental analysis was performed on a Fisons - EA-1108 CHNS-O Element Analyzer (Table 1).

All test microorganisms were received from the Refik Saydam Hıfzıssıhha Institute (Ankara, Turkey). Those are *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 60193, *Candida tropicalis* ATCC 13803 and *Bacillus cereus* 709 ROMA. All chemicals have been weighed and then dissolved in dimethyl sulfoxide (DMSO), as solvent, to prepare extract stock solutions of 10 mg/mL (Table 2).

The ager-well diffusion method was used for the simple susceptibility screening test (Mullican et al., 1993) as adapted in the previously reported method (Tozkopran et al., 2005). All microorganisms were suspended in Mueller-Hinton (Difco, Detroit, MI, USA) broth and diluted to ca. 10^6 colony-forming units (CFU) per mL. They were flood-inoculated on the surface of Mueller Hinton agar and Sabouraud dextrose agar (SDA) (Difco), then they were dried. SDA was used for *C. albicans* and *C. tropicalis.* 5-mm diameter wells were cut using a sterile cork-borer from the agar and 500 μg/50 μL (10 mg/mL) of the chemical substances were transferred into the wells. The plates were then incubated for about 18 h at 35 ºC. The antimicrobial activity was determined by measuring the inhibition zone against the test organism. Ampicillin (10 μg/50 μL) was used as the control antibiotic. Triflucan (5 μg/50 μL) was used as control fungicide. DMSO was used as the control solvent. The results are shown in Table 2.

Table 1. Compounds and R groups

Compound No.	R group
4a, 5a	Br
4b, 5b	Cl
4c, 5c	OCH_3

Synthesis of benzoic acid hydrazide (1)

Methyl benzoate (0.01 mole, 1.63g, 15 mL) with hydrazine hydrate (0.01 mole, 0.6g, 0.58 mL) was refluxed (reflux time was 1 hour, later 40 mL absolute ethanol was added then reflux was continued for 3 more hours. After cooling the solution, white crystals were formed which were then recrystallized by ethanol. Yield was 1.03g, and 75.73%. melting point (M.p), 112-114°C (Selvaraj et al., 2011)

Synthesis of potassium dithiocarbazinate (2)

Potassium hydroxide (0.03 mole, 1.68 g) and acid hydrazide, which is 1, (0.01 mole, 1.36g) mixture was dissolved in absolute ethyl alcohol (15 mL). The solution was then cooled in an ice bath and carbon disulfide (0.05 mole, 3mL) was added in small portions with continued stirring. Then the reaction mixture was allowed to continue stirring at room temperature for 18 hours. Dry ether (10mL) was then added to the solution, which resulted in forming a yellow precipitate, which was filtered and washed

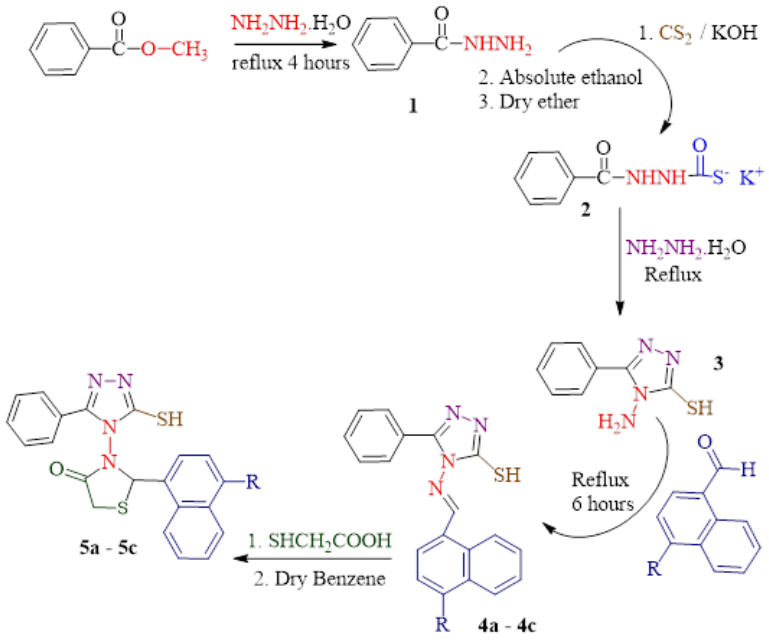

Figure 1. The Steps for Synthesis of Compounds (1-5)

using ether to obtain dried potassium salt (2) which was used in the next step without further purification. Yield was 1.68 g, and67.20%., M.p. 186-188°C (Selvaraj et al., 2011).

Synthesis of 4-amino-5-phenyl-4H-1, 2, 4-triazole -3-thiol (3)

5g (0.02 mole) of potassium salt (2) was dissolved in 40 mL of water and hydrazine hydrate (2mL, 0.04 mole) was added to the suspension, the color of the reaction mixture went from yellow to green, after the mixture was refluxed until the evaluation of hydrogen sulfide was observed and which was ceased by lead acetate paper. Then the reaction mixture was allowed to cool at room temperature and diluted with 30 mL of cold water. Upon acidification by HCl, white powder was obtained as a precipitate, which was then recrystallized by ethanol. Yield was 1.25 g and 65.10%., M.p. 198-200°C (Selvaraj et al., 2011).

Synthesis of Schiff bases (4a-c)

A mixture of compound (3) (0.01mole) and respective aromatic aldehyde (0.01mole) was refluxed for 4 to 6 hours in absolute ethanol (25 mL) and a few drops of glacial acetic acid. The reaction mixture was then cooled, and the precipitate formation occurred which was filtered and recrystallized by using ethanol. (Selvaraj et al., 2011; Čačić, Molnar, Šarkanj, Has-Schön, & Rajković, 2010)

(E)-4-(4-bromonaphthalen-1-yl methylene amino)-5-phenyl-4H-1,2,4-triazole-3-thiol (4a)

Yield 2.91g, 71.15%. M.p. 173-175 °C; IR (KBr) cm^{-1} 3109 (ν aromatic C-H), 2928 (ν aliphatic C-H), 2740 (ν S-H), 1616 (ν C=N); ^{1}H-NMR (DMSO-d_6) δ (ppm) Ar-H [7.28 (d, 1H, j= 8.83 Hz), 7.40 (d, 1H, j= 8.83 Hz), 7.50-7.75 (m, 5H), 7.90 (d, 1H, j= 8.65 Hz), 7.94-8.10 (m, 2H), 8.16 (d, 1H, j= 8.65 Hz)], 9.31 (s, 1H, N=CH), 12.80 (s, 1H, SH); ^{13}C-NMR (DMSO-d_6) δ (ppm) 171.52 (N=CH), 152.07, 150.76 (2C, triazole C_3, C_5), Ar-C [150.20 (2CH), 148.72 (C), 139.48 (C), 133.93 (C), 129.19 (2CH), 128.60 (2CH), 125.35 (CH), 123.34 (C), 122.46 (C), 121.19 (2CH), 115.90 (CH), 110.86 (CH)]; Elemental analysis ($C_{19}H_{13}BrN_4S$); calcd. C, 55.75; H, 3.20; N, 13.68; S, 7.83; Found C, 55.62; H, 3.27; N, 13.41; S, 8.06%.

(E)-4-(4-chloronaphthalen-1-yl methylene amino)-5-phenyl-4H-1,2,4-triazole-3-thiol (4b)

Yield 2.48g, 7.94%). M.p. 197-199 °C; IR (KBr) cm^{-1} 3112 (ν aromatic C-H), 2933 (ν aliphatic C-H), 2744 (ν S-H), 1621 (ν C=N); ^{1}H-NMR ($DMSO_6$) δ (ppm) Ar–H [7.45 (d, 2H, j= 8.65 Hz), 7.60-7.70 (m, 2H), 7.75-7.88 (m, 4H), 7.90-8.00 (m, 3H)], 8.80 (s, 1H, N=CH), 12.74 (s, 1H, SH); ^{13}C-NMR (DMSO-d_6) δ (ppm) 169.97 (N=CH), 150.00, 149.80 (2C, triazole C_3, C_5), Ar-C [140.11 (C), 139.98 (C), 130.22 (C), 130.09 (CH), 129.85 (2CH), 129.63 (2CH), 129.00 (2CH), 127.38 (2CH), 126.41 (C), 124.00 (2CH), 123.56 (C)]; Elemental analysis ($C_{19}H_{13}ClN_4S$); calcd. C, 62.54; H, 3.59; N, 15.33; S, 8.78; Found C, 62.71; H, 3.52; N, 15.41; S, 8.14%.

(E)-4-(4-methoxynaphthalen-1-yl) methylene amino)-5-phenyl-4H-1,2,4-triazole-3-tiol (4c)

Yield 2.62g, 72.77%. M.p. 227-229 °C; IR (KBr) cm^{-1} 3107 (ν aromatic C-H), 2968 (ν aliphatic C-H), 2740 (ν S-H), 1616 (ν C=N); ^{1}H-NMR (DMSO-d6) δ (ppm) 3.78 (s, 3H, OCH_3), Ar–H [6.80 (d, 1H, j= 8.83 Hz), 7.14 (d, 1H, j= 8.83 Hz), 7.20-7.40 (m, 2H), 7.45-7.55 (m, 3H), 7.60-7.70 (m, 2H), 7.72-7.85 (m, 2H), 8.44 (s, 1H, N=CH), 13.02 (s, 1H, SH); ^{13}C-NMR (DMSO−6) δ (ppm) 171.92 (N=CH), 150.66, 150.17 (2C, triazole C_3, C_5), Ar–C [148.33 (C), 139.21 (C), 129.57 (CH), 129.31 (2CH), 128.89 (2CH), 127.96 (2CH), 127.18 (2CH), 126.60 (C), 125.75 (CH), 123.12 (C), 122.72 (C), 115.79 (CH)], 55.46 (OCH_3); Elemental analysis ($C_{20}H_{16}N_4SO$); calcd. C, 66.65; H, 4.47; N, 15.54; S, 8.89; Found C, 66.72; H, 4.39; N, 15.59; S, 8.47%.

Synthesis of thiazolidenon derivatives (5a-c)

Schiff bases (4a-c) (0.002 mole) mixture with thioglycolic acid (0.04 mole, 0.26 mL) in the presence of dry benzene (30 mL) refluxed for 10 hours. Then, the mixture was concentrated and recrystallized with ethanol (Selvaraj et al., 2011).

2-(4-bromonaphthalen-1-yl)-3-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)thiazolid- in-4-one (5a)

Yield 3.61g, 77.28%. M.p. 162-164°C; IR (KBr) cm^{-1} 3018 (ν aromatic C-H), 2913 (ν aliphatic C-H), 2748 (ν S-H), 1718 (C=O), 1609 (ν C=N), 694 (C-S-C); 1H-NMR (DMSO-d_6) δ (ppm) 3.91 (s, 2H, CH_2), 5.48 (s, ^{1}H, CH), Ar–H [7.30 (d, 2H, j= 8.65 Hz), 7.40-7.60 (m, 2H), 7.65-7.70 (m, 2H), 7.84-7.91 (m, 1H), 8.20- 8.45 (m, 4H)], 14.03 (s, 1H, SH); ^{13}C-NMR (DMSO-d_6) δ (ppm) 165.14 (C=O), 149.37, 148.32 (2C, triazole C3, C5), Ar–C [161.96 (2C), 139.25 (2C), 133.13 (2CH), 132.96 (2CH), 131.10 (2CH), 129.41 (2CH), 127.06 (2CH), 124.65 (C),113.41 (CH)], 55.26 (CH), 45.87 (CH_2); Elemental analysis ($C_{20}H_{15}BrN_4S_2O$); calcd. C, 50.96; H, 3.20; N, 11.88; S, 13.60; O, 3.39; Found C, 50.72; H, 3.22; N, 11.51; S, 13.35; O, 3.81%.

2-(4-chloronaphthalen-1-yl)-3-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4 yl)thiazolidi-n-4-one (5b)

Yield 2.80g, 65.57%. M.p. 181-183°C; IR (KBr) cm^{-1} 3010 (ν aromatic C-H), 2945 (ν aliphatic C-H), 2698 (ν S-H), 1702 (C=O), 1614 (ν C=N), 672 (C-S-C); 1H-NMR (DMSO-d_6) δ (ppm) 3.80 (s, 2H, CH_2), 5.69 (s, 1H, CH), Ar–H [6.60 (d, 1H, j= 8.83 Hz), 7.28 (bs, 1H), 7.60-7.80 (m, 5H), 8.00-8.20 (m, 4H)], 13.67 (s, 1H, SH); ^{13}C-NMR (DMSO-d_6) δ (ppm) 158.68 (C=O), 151.12, 149.78 (2C, triazole C_3, C_5), Ar–C [139.08 (2C), 134.01 (CH), 133.82 (CH), 130.23 (2CH), 129.65 (2CH), 127.82 (2CH), 124.66 (C), 121.95 (CH), 118.04 (2C), 115.10 (2CH)], 58.92 (CH), 45.18 (CH_2); Elemental analysis ($C_{20}H_{15}ClN_4S_2O$); calcd. C, 56.26; H, 3.54; N, 13.12; S, 15.01; O, 3.74; Found C, 56.12; H, 3.46; N, 13.85; S, 15.18; O, 3.65%.

3-(3-mercapto-5-phenyl-4H-1,2,4-triazol-4-yl)-2-(4-methoxynaphthalen-1-yl)thiazol-idin-4-one (5c)

Yield 3.40g, 80.57%). M.p. 204-206°C; IR (KBr) cm^{-1}3032(ν aromatic C-H), 2957 (ν aliphatic C-H), 2679 (ν S-H), 1715 (C=O), 1597 (ν C=N), 671 (C-S-C); ^{1}H-NMR (DMSO-d_6) δ (ppm) 3.65 (s, 3H, OCH_3), 3.96 (s, 2H, CH_2), 5.31 (s, 1H, CH), Ar–H [6.91 (d, 1H, j= 8.83 Hz), 7.24, (d, 1H, j= 8.83 Hz), 7.38-7.70 (m, 5H), 7.80-8.00 (m, 4H)], 13.18 (s, 1H, SH); ^{13}C-NMR (DMSO-d_6) δ (ppm) 164.05 (C=O), 151.05 149.82 (2C, triazole C_3, C_5), Ar–C [139.83 (2C), 131.84 (2CH), 130.56 (2CH), 130.17 (CH), 129.98 (2CH), 129.41 (2CH), 128.53 (2CH), 127.00 (C), 124.27 (C), 124.05 (C)], 59.06 (CH)], 55.82 (OCH3), 45.12 (CH_2); Elemental analysis ($C_{21}H_{18}N_4S_2O$); calcd. C, 59.69; H, 4.29; N, 13.25; S, 15.17; O, 7.57; Found C, 59.47; H, 4.24; N, 13.29; S, 15.23; O, 7.64%.

RESULTS AND DISCUSSION

The synthesis of basic 4-amino-5-phenyl-4H-1, 2, 4-triazole-3-thiol **(3)** nucleus was carried out as in the literature method (Fedotov & Hotsulia). Then compound **(3)** was used for the synthesis of Schiff bases **(4a-c)**, which showed confirmation by the absence of NH_2 peak in IR spectra and the presence of peaks at (8.92-9.20) ppm due to N=CH in 1HNMR spectra and singlet as expected in all three compounds. The proton bound to the azomethine group is generally resonance in the range of δ=8-9 ppm. IR spectra showed the C=N bands of **(4a–c)** in the 1616-1621 cm−1 area. Peaks of imine carbons are seen in the 13C-NMR spectrum between δ=164-168 ppm. Imine peak emerged as a singlet. It was observed that the NMR results supported the formation of the compound and were consistent with the literature (Fedotov & Hotsulia, 2023; Valicsek & Badea, 2021; Klimesova, Zahajska, Waisser, Kaustova, & Mollmann, 2004).

The reaction of Schiff bases **(4a-c)** with thioglycolic acid in dry benzene resulted in the formation of thiazolidenone derivatives **(5a-c) (Figure 1)**. The FTIR spectrum of compounds (5a-c) confirmed by the presence of stretching band between 1718-1702 $cm^{-1}$for C=O of thiazolidinone ring and absorption bands at 671-694 cm^{-1} due to C-S-C, 3010-3032 cm^{-1} for C-H aromatic, 2913-2957 cm^{-1} For C-H aliphatic, 2679-2748 for S-H group and 1614-1597 cm^{-1} for C=N of triazole ring.

^{1}HNMR spectrum shows the disappearance of the azomethine group (CH=N) and the appearance of a signal at 3.42-3.87 ppm due to the methylene group ($COCH_2S$) singlet as expected in all three compounds. Singlet signal at 5.31-5.64 ppm for CH (SCHN), singlet signal at 13-14 ppm for S-H group, and

Table 2. Antimicrobial activity screening result for the selected compounds dissolved in dimethyl sulfoxide (DMSO) as solvent (10 mg/mL)

Compound no.	**Microorganisms and inhibition zone (mm)**								
	Ec	Pa	Yp	Kp	Ef	Sa	Bc	Ca	Ct
4a	5	14	5	13	5	5	5	5	15
4b	5	23	5	18	5	5	5	22	20
4c	5	5	5	5	5	10	5	25	17
5a	5	13	5	5	5	13	5	5	5
5b	5	5	5	5	5	12	5	5	5
5c	5	5	5	5	5	5	5	5	11
DMSO	5	5	5	5	5	5	5	5	5
Ampicillin	8	5	5	5	12	16	13	5	5
Triflucan								25	25

-: Results were concluded based on the inhibition zone diameter (5 mm: no antimicrobial activity; > 5 mm: positive antimicrobial activity). Ec: *Escherichia coli* ATCC 25922; Pa: *Pseudomonas aeruginosa* ATCC 10145; Yp: *Yersinia pseudotuberculosis* ATCC 911; Kp: *Klebsiella pneumonia* ATCC 13883; Ef: *Enterococcus faecalis* ATCC29212; Sa: *Staphylococcus aureus* ATCC 25923; Bc: *Bacillus cereus* 709 ROMA; Ca: *Candida albicans* ATCC 60193; Ct: *Candida tropicalis* ATCC 13803.

signal 3.76 ppm for (OCH_3) group as compound 5c. The peaks of carbonyl, CH_2, and CH carbons are seen in the ^{13}C- NMR spectrum between δ = 156-157, 5.31-5.64, 3.42-3.87 ppm respectively. It was observed that the NMR results supported the formation of the compound and were consistent with the literature (Fedotov &Hotsulia, 2023; Valicsek & Badea, 2021; Klimesova et al., 2004).

Compound **5c** showed good antifungal activity only against yeast-like fungi, while compound **4a-c** showed antimicrobial activity against bacteria and yeast-like fungi. Compound **5b** was only found effective on the gram-positive bacteria, *S. aureus* ATCC 25923. The highest activity was observed against *P. aeruginosa* ATCC 10145 by **4b**. Compound **5a** was found to be effective on both, *S. aureus* ATCC 25923 as well as *P. aeruginosa* ATCC 10145. Compound **4c** showed the highest activity against *Candida albicans* ATCC 60193.

CONCLUSION

In summary, compounds (4a-c, 5a-c) were successfully synthesized and characterized quantitatively and qualitatively by using FTIR, 1HNMR, 13CNMR, and elemental analysis. 4-amino-5-phenyl-4H-1,2,4-triazole-3-thiol derivatives and their promising antimicrobial activities were proved.

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ORCID IDs of the authors

Nurhan Gumrukcuoglu 0000-0002-9669-6318
Muhammad Imran 0000-0003-1795-1040
Inam Iqbal 0009-0001-5891-8477

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