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SYNTHESIS OF SOME N-(BENZOTHIAZOL-2-YL)-2-(4H-[1,2,4]TRIAZOL -3-YLSULFANYL)ACETAMIDES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

The aim of this study is to synthesize novel benzothiazolyl-amide derivatives and investigate their antimicrobial activities. N-(Benzothiazol-2-yl)-2-(4H-[1,2,4]triazol-3-ylsulfanyl) acetamide derivatives (**3a-j**) were prepared by the reaction of N-(benzothiazol-2-yl)-2-chloroacetamides (**1a-e**) with appropriate 4H-[1,2,4]triazol-2-thione (**2a-b**). The chemical structures of the compounds were elucidated by elemental analyses, IR, ^{1}H-NMR, ^{13}C-NMR and FAB^{+}-MS spectral data. Their antimicrobial activities against *Escherichia coli* (NRRL B-3008), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (NRRL B-123), *Salmonella typhimurium* (ATCC 13311), Methicillin-resistant *Staphylococcus aureus* (MRSA) (clinic isolate), *Candida albicans* (NRRL Y-12983) and *Candida parapsilosis* (NRRL Y-12696) were investigated. The results showed that all of the tested compounds were inactive against the test organisms.

Keywords: Benzothiazole, Triazole, Antimicrobial activity.

BAZI N-(BENZOTİYAZOL-2-İL)-2-(4H-[1,2,4]TRİAZOL-3-İLSÜLFANİL)ASETAMİDLERİN SENTEZİ VE BUNLARIN ANTİMİKROBİYAL AKTİVİTELERİ

ÖZ

Bu çalışmanın amacı, yeni benzotiyazolil-amid türevlerini sentezlemek ve bunların antimikrobiyal aktivitelerini araştırmaktır. N-(benzotiyazol-2-il)-2-kloroasetamidler ile uygun 4H-[1,2,4]triazol-2-tiyonların reaksiyona sokulmasıyla N-(Benzotiyazol-2-il)-2-(4H-[1,2,4]triazol-3-ilsülfanil) asetamid türevleri hazırlanmıştır. Sentezlenen bileşiklerin kimyasal yapıları elemental analiz, IR, ^{1}H-NMR, ^{13}C-NMR ve FAB^{+}-MS spektral verileri ile aydınlatılmıştır. Bunların antimikrobiyal aktiviteleri *Escherichia coli* (NRRL B-3008), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (NRRL B-123), *Salmonella typhimurium* (ATCC 13311), Methicillin-resistant *Staphylococcus aureus* (MRSA) (clinic isolate), *Candida albicans* (NRRL Y-12983) ve *Candida parapsilosis*'e (NRRL Y-12696) karşı araştırılmıştır. Sonuçlar test edilen tüm bileşiklerin, test organizmalarına karşı inaktif olduğunu gösterdi.

Anahtar Kelimeler: Benzotiyazol, Triazol, Antimikrobiyal aktivite.

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1. INTRODUCTION

During the past decades, the problem of multidrug resistant microorganisms has reached on alarming level around the world. Due to this reason, novel classes of antibacterial agents with new mechanisms are crucial need to combat with the multidrug-resistant infections. In the past years, some azole derivatives were developed as novel antimicrobial agents, for example, Linezolid and Eperezolid are currently used for the treatment of multidrug-resistant Gram-positive infections (Bayrak et al. 2009).

The chemistry of 1,2,4-triazoles has received noteworthy attention because of their synthetic and biological significance. Derivatives of 1,2,4-triazole have been found to have difference pharmacological activity, for example fungicidal, bactericidal, anti-tumor, anti-inflammatory, insecticidal, and herbicidal, among others (Swamy et al. 2006). A large number of 1,2,4-triazole-containing ring systems, for instance Fluconazole, Itraconazole, Voriconazole, Ravuconazole and Posaconazole have been incorporated into a wide variety of therapeutically interesting drug candidates with, for example, anti-inflammatory, CNS-stimulant, sedative, antianxiety, antimicrobial, and antimycotic activity (Shaker 2006). Furthermore, there are known drugs containing the 1,2,4-triazole group (Saadeh et al. 2010), including triazolam, alprazolam, and etizolam. In addition, thione-substituted 1,2,4-triazoles and their derivatives (Shaker 2006; Saadeh et al. 2010) have been found to have a variety of biological activity, for instance antibacterial, antifungal, antitubercular, antimycobacterial, anticancer, diuretic, and hypoglycemic properties.

Similarly, benzothiazoles are an important class of privileged structures of medicinal importance owing to their recognized biological and therapeutic activities (Bose and Idrees 2010). As such, these heterocycles constitute key structural motifs that show a wide range of biological properties, such as imaging agents for β-amyloid plaques (Henriksen et al. 2007), inhibitors of stearoyl-coenzyme A δ-9 desaturase (Black et al. 2006), antitumor (Hutchinson et al. 2001), antimicrobial (Palmer et al. 1971) agents, and Gram-positive selective antibacterials (Ali et al. 2001). After extensive literature search, it was observed that, till date enough effort has not been made to combine this two moieties as a single molecular scaffold and to identify novel candidates that may be value in designing new antimicrobial agent. In view of this data, we reported the synthesis of new 1,2,4-triazoles incorporated with benzothiazole which possessed wide variety of biological activity encouraging antimicrobial activity.

2. EXPERIMENTAL

2.1 *Chemistry*

All chemicals were obtained from Aldrich Chemical Co. (Steinheim, Germany). All melting points (m.p.) were determined in open capillaries on a Gallenkamp apparatus (Weiss-Gallenkamp, Loughborough-United Kingdom) and are uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel 60G (Merck, Darmstadt-Germany). Elemental analyses were performed on a Perkin Elmer EAL 240 elemental analyser. Spectroscopic data were recorded with the following instruments: ^{1}H-NMR: Bruker 400 MHz spectrometer and ^{13}C-NMR Bruker 100 MHz spectrometer (Bruker, Billerica, Massachusetts, USA) in DMSO-d_6 using TMS as internal standard; and MS-FAB: VG Quattro Mass spectrometer (Agilent, Minnesota, USA).

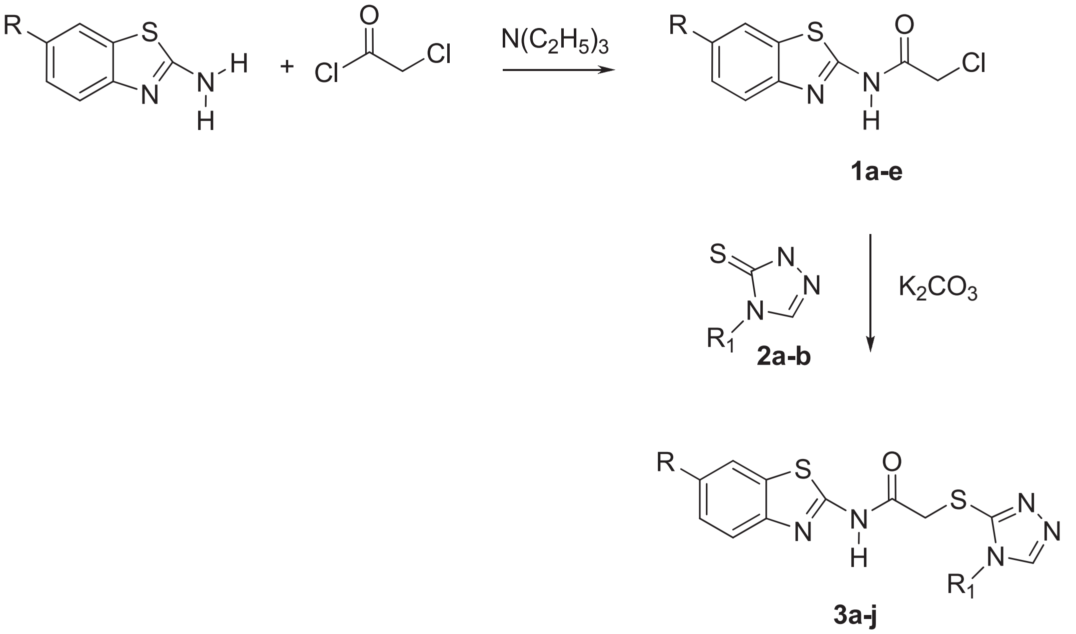
2.1.1 General Procedure for Synthesis of the Compounds

N-(benzothiazol-2-yl)-2-chloroacetamides ***(1a-e)***

Chloroacetyl chloride (0,1 mol) was added dropwise with stirring to a mixture of 2-aminobenzothiazole/6-substituted-2-aminobenzothiazole (0,1 mol) and triethylamine in toluene at 0-5 °C. The solvent was evaporated under reduced pressure. The residue was washed with water to remove triethylamine hydrochloride and crystallized from ethanol (Bhargava and Ram 1965).

N-(Benzothiazol-2-yl)-2-(4H-[1,2,4]triazol-3-ylsulfanyl) acetamides ***(3a-j)***

A mixture of N-(benzothiazol-2-yl)-2-chloroacetamides **(1a-e)** (0.015 mol), appropriate 4H-[1,2,4]triazol-2-thione (**2a-b)** (0.015 mol) and K_2CO_3 (0.015 mol) in acetone (50 mL) was refluxed for 10-12 h. After cooling to r.t., the solution was evaporated to dryness. The residue was washed with water (200 mL) and recrystallized from ethanol (Scheme 1). Some characteristics of the synthesized compounds are shown in Table 1.



Scheme 1. Synthetic pathway for the preparation of compounds **3a-j**.

Table 1. Some characteristics of the compounds **3a-j**

Comp.	R	R$_1$	Molecular Formula	M.W.	M.P. (^{o}C)	Yield (%)
3a	H	H	$C_{11}H_9N_5OS_2$	291	143-146	68
3b	Cl	H	$C_{11}H_8ClN_5OS_2$	325	118-120	72
3c	CH$_3$	H	$C_{12}H_{11}N_5OS_2$	305	170-172	64
3d	OCH$_3$	H	$C_{12}H_{11}N_5O_2S_2$	321	188-190	63
3e	OC$_2$H$_5$	H	$C_{13}H_{13}N_5O_2S_2$	335	156-158	66
3f	H	CH$_3$	$C_{12}H_{11}N_5OS_2$	305	229-230	62
3g	Cl	CH$_3$	$C_{12}H_{10}ClN_5OS_2$	339	259-261	69
3h	CH$_3$	CH$_3$	$C_{13}H_{13}N_5OS_2$	319	240-242	65
3i	OCH$_3$	CH$_3$	$C_{13}H_{13}N_5O_2S_2$	335	223-225	60
3j	OC$_2$H$_5$	CH$_3$	$C_{14}H_{15}N_5O_2S_2$	349	220-221	67

3a: **IR [ν, cm^{-1}, KBr]:** 3353 (amine N-H stretching), 3216 (amide N-H stretching), 3056 (aromatic C-H stretching), 2924 (aliphatic C-H asymmetric stretching), 2847 (aliphatic C-H symmetric stretching), 1684 (amide C=O stretching), 1598, 1552, 1444 (C=N and C=C stretching), 1330, 1261, 1172, 1015 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 4.22 (2H, s, S-CH$_2$), 5.87 (1H, s, triazole N-H), 7.29 (1H, t, J = 7.7 Hz, benzothiazole H$_6$), 7.43 (1H, t, J = 7.7 Hz, benzothiazole H$_5$), 7.74 (1H, d, J = 8.0 Hz, benzothiazole H$_7$), 7.94 (1H, d, J = 8.0 Hz, benzothiazole H$_4$), 8.55 (1H, s, triazole H$_5$), 12.61 (1H, m, N-H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 34.67 (CH$_2$), 120.53 (CH), 121.58 (CH), 123.46 (CH), 125.87 (C), 131.49 (C), 146.35 (CH), 148.54 (C), 150.41 (C), 156.69 (C), 167.50 (C). **MS-FAB$^+$**: m/z: 292 [M + 1]. Anal. Calcd for $C_{11}H_9N_5OS_2$: C, 45.35; H, 3.11; N, 24.04. Found: C, 45.33; H, 3.13; N, 24.05.

3b: **IR [ν, cm^{-1}, KBr]:** 3334 (amine N-H stretching), 3194 (amide N-H stretching), 3065 (aromatic C-H stretching), 2922 (aliphatic C-H asymmetric stretching), 2850 (aliphatic C-H symmetric stretching), 1684 (amide C=O stretching), 1605, 1554, 1440 (C=N and C=C stretching), 1335, 1256, 1173, 1089 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ 4.26 (2H, s, S-CH$_2$), 5.94 (1H, s, triazole N-H), 7.45 (1H, d, *J* = 8.0 Hz, benzothiazole H$_5$), 7.75 (1H, d, *J* = 8.0 Hz, benzothiazole H$_7$), 8.11 (1H, s, benzothiazole H$_4$), 8.52 (1H, s, triazole H$_5$), 12.85 (1H, m, N-H). **^{13}C NMR** (100 MHz, DMSO-*d*$_6$): δ 34.58 (CH$_2$), 121.39 (CH), 121.73 (CH), 126.48 (CH), 127.69 (C), 130.93 (C), 147.41 (CH), 150.46 (C), 155.98 (C), 158.73 (C), 167.89 (C). **MS-FAB$^+$**: m/z: 324 [M-1], 326 [M + 1], 327 [M + 2]. Anal. Calcd for $C_{11}H_8ClN_5OS_2$: C, 40.55; H, 2.48; N, 21.50. Found: C, 40.47; H, 2.54; N, 21.49.

3c: **IR [ν, cm^{-1}, KBr]:** 3346 (amine N-H stretching), 3191 (amide N-H stretching), 3064 (aromatic C-H stretching), 2926 (aliphatic C-H asymmetric stretching), 2857 (aliphatic C-H symmetric stretching), 1675 (amide C=O stretching), 1608, 1565, 1458 (C=N and C=C stretching), 1335, 1255, 1175, 1055 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ 2.40 (3H, s, CH$_3$), 4.25 (2H, s, S-CH$_2$), 5.93 (1H, s, triazole N-H), 7.26 (1H, d, *J* = 8.3 Hz, benzothiazole H$_5$), 7.65 (1H, d, *J* = 8.3 Hz, benzothiazole H$_7$), 7.75 (1H, s, benzothiazole H$_4$), 8.53 (1H, s, triazole H$_5$), 12.60 (1H, m, N-H). **^{13}C NMR** (100 MHz, DMSO-*d*$_6$): δ 20.95 (CH$_3$), 34.56 (CH$_2$), 120.24 (CH), 121.27 (CH), 127.46 (CH), 130.95 (C), 131.59 (C), 146.47 (CH), 150.67 (C), 155.95 (C), 156.85 (C), 167.45 (C). **MS-FAB$^+$**: m/z: 306 [M + 1]. Anal. Calcd for $C_{12}H_{11}N_5OS_2$: C, 47.20; H, 3.63; N, 22.93. Found: C, 47.28; H, 3.61; N, 22.97.

3d: **IR [ν, cm^{-1}, KBr]:** 3355 (amine N-H stretching), 3189 (amide N-H stretching), 3083 (aromatic C-H stretching), 2930 (aliphatic C-H asymmetric stretching), 2859 (aliphatic C-H symmetric stretching), 1676 (amide C=O stretching), 1605, 1582, 1462 (C=N and C=C stretching), 1334, 1257, 1148, 1061 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ 3.80 (3H, s, OCH$_3$), 4.20 (2H, s, S-CH$_2$), 5.94 (1H, s, triazole N-H), 7.03 (1H, dd, *J* = 8.8, 2.6 Hz, benzothiazole H$_5$), 7.57 (1H, d, *J* = 2.6 Hz, benzothiazole H$_7$), 7.65 (1H, d, *J* = 8.8 Hz, benzothiazole H$_4$), 8.49 (1H, m, triazole H$_5$), 13.00 (1H, m, N-H). **^{13}C NMR** (100 MHz, DMSO-*d*$_6$): δ 34.80 (CH$_2$), 55.52 (CH$_3$), 104.47 (CH), 115.09 (CH), 121.21 (CH), 132.55 (C), 142.17 (C), 150.69 (C), 155.80 (CH), 156.14 (C), 156.61 (C), 167.53 (C). **MS-FAB$^+$**: m/z: 322 [M + 1]. Anal. Calcd for $C_{12}H_{11}N_5O_2S_2$: C, 44.85; H, 3.45; N, 21.79. Found: C, 44.81; H, 3.51; N, 21.87.

3e: **IR [ν, cm^{-1}, KBr]:** 3350 (amine N-H stretching), 3182 (amide N-H stretching), 3058 (aromatic C-H stretching), 2926 (aliphatic C-H asymmetric stretching), 2853 (aliphatic C-H symmetric stretching), 1683 (amide C=O stretching), 1600, 1584, 1452 (C=N and C=C stretching), 1335, 1220, 1158, 1045 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ 1.34 (3H, t, *J* = 7.0 Hz, benzothiazole O-CH$_2$-**CH$_3$**), 4.05 (2H, t, *J* = 7.0 Hz, benzothiazole O-**CH$_2$**-CH$_3$), 4.18 (2H, s, S-CH$_2$), 5.95 (1H, s, triazole N-H), 7.01 (1H, dd, *J* = 8.8, 2.6 Hz, benzothiazole H$_5$), 7.53 (1H, d, *J* = 2.6 Hz, benzothiazole H$_7$), 7.63 (1H, d, *J* = 8.8 Hz, benzothiazole H$_4$), 8.46 (1H, s, triazole H$_5$), 12.63 (1H, m, N-H). **^{13}C NMR** (100 MHz, DMSO-*d*$_6$): δ 14.43 (CH$_3$), 34.84 (CH$_2$), 63.69 (CH$_2$), 105.08 (CH), 115.41 (CH), 121.18 (CH), 132.53 (C), 142.08 (C), 145.88 (CH), 155.34 (C), 155.81 (C), 156.67 (C), 167.55 (C). **MS-FAB$^+$**: m/z: 336 [M + 1]. Anal. Calcd for $C_{13}H_{13}N_5O_2S_2$: C, 46.55; H, 3.91; N, 20.88. Found: C, 46.64; H, 3.93; N, 20.87.

3f: **IR [ν, cm^{-1}, KBr]:** 3350 (amine N-H stretching), 3213 (amide N-H stretching), 3054 (aromatic C-H stretching), 2925 (aliphatic C-H asymmetric stretching), 2842 (aliphatic C-H symmetric stretching), 1683 (amide C=O stretching), 1602, 1555, 1447 (C=N and C=C stretching), 1334, 1264, 1174, 1017 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ 3.61 (3H, s, CH$_3$), 4.22 (2H, s, S-CH$_2$), 7.32 (1H, t, *J* = 7.2 Hz, benzothiazole H$_6$), 7.45 (1H, t, *J* = 7.2 Hz, benzothiazole H$_5$), 7.77 (1H, d, *J* = 8.0 Hz, benzothiazole H$_7$), 7.98 (1H, d, *J* = 8.0 Hz, benzothiazole H$_4$), 8.58 (1H, s, triazole H$_5$), 12.67 (1H, bs, N-H). **^{13}C NMR** (100 MHz, DMSO-*d*$_6$): δ 30.80 (CH$_2$), 36.37 (CH$_2$), 120.63 (CH), 121.73 (CH), 123.66 (CH), 126.17 (CH), 131.41 (C), 146.35 (CH), 148.33 (C), 148.45 (C), 157.64 (C), 167.24 (C). **MS-FAB$^+$**: m/z: 306 [M + 1]. Anal. Calcd for $C_{12}H_{11}N_5OS_2$: C, 47.20; H, 3.63; N, 22.93. Found: C, 47.36; H, 3.53; N, 22.92.

3g: **IR [ν, cm^{-1}, KBr]:** 3343 (amine N-H stretching), 3200 (amide N-H stretching), 3058 (aromatic C-H stretching), 2920 (aliphatic C-H asymmetric stretching), 2848 (aliphatic C-H symmetric stretching), 1683 (amide C=O stretching), 1604, 1557, 1443 (C=N and C=C stretching), 1336, 1262, 1172, 1087 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-*d*$_6$): δ 3.61 (3H, s, CH$_3$), 4.22 (2H, s, S-CH$_2$), 7.47 (1H, d, *J* = 8.6 Hz, benzothiazole H$_5$), 7.75 (1H, d, *J* = 8.6 Hz, benzothiazole H$_7$), 8.13 (1H, bs, benzothiazole H$_4$), 8.57 (1H, s, triazole H$_5$), 12.76 (1H, m, N-H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 30.79 (CH_3), 36.30 (CH_2), 121.46 (CH), 121.85 (CH), 126.53 (CH), 127.70 (C), 133.12 (C), 146.35 (CH), 147.36 (C), 148.31 (C), 158.53 (C), 167.49 (C). **MS-FAB$^+$**: m/z: 338 [M-1], 340 [M + 1], 341 [M + 2]. Anal. Calcd for $C_{12}H_{10}ClN_5OS_2$: C, 42.41; H, 2.97; N, 20.61. Found: C, 42.37; H, 2.91; N, 20.59.

3h: **IR [ν, cm^{-1}, KBr]:** 3339 (amine N-H stretching), 3187 (amide N-H stretching), 3064 (aromatic C-H stretching), 2922 (aliphatic C-H asymmetric stretching), 2851 (aliphatic C-H symmetric stretching), 1684 (amide C=O stretching), 1608, 1565, 1462 (C=N and C=C stretching), 1331, 1262, 1170, 1027 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 2.41 (3H, s, benzothiazole CH_3), 3.61 (3H, s, CH_3), 4.20 (2H, s, S-CH_2), 7.25 (1H, d, J = 8.3 Hz, benzothiazole H_5), 7.64 (1H, d, J = 8.3 Hz, benzothiazole H_7), 7.76 (1H, bs, benzothiazole H_4), 8.58 (1H, s, triazole H_5), 12.58 (1H, m, N-H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.95 (CH_3), 30.79 (CH_3), 36.34 (CH_2), 120.28 (CH), 121.31 (CH), 127.49 (CH), 131.55 (C), 133.15 (C), 146.34 (CH), 146.44 (C), 148.34 (C), 156.73 (C), 167.06 (C). **MS-FAB$^+$**: m/z: 320 [M + 1]. Anal. Calcd for $C_{13}H_{13}N_5OS_2$: C, 48.89; H, 4.10; N, 21.93. Found: C, 48.66; H, 3.93; N, 22.02.

3i: **IR [ν, cm^{-1}, KBr]:** 3319 (amine N-H stretching), 3194 (amide N-H stretching), 3068 (aromatic C-H stretching), 2920 (aliphatic C-H asymmetric stretching), 2849 (aliphatic C-H symmetric stretching), 1682 (amide C=O stretching), 1610, 1563, 1463 (C=N and C=C stretching), 1333, 1258, 1167, 1057 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 3.60 (3H, s, CH_3), 3.81 (3H, s, benzothiazole OCH_3), 4.21 (2H, s, S-CH_2), 7.05 (1H, d, J = 8.6 Hz, benzothiazole H_5), 7.55 (1H, s, benzothiazole H_7), 7.66 (1H, d, J = 8.6 Hz, benzothiazole H_4), 8.46 (1H, s, triazole H_5), 12.43 (1H, m, N-H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 20.96 (CH_3), 34.56 (CH_2), 55.59 (CH_3), 104.28 (CH), 114.38 (CH), 121.59 (CH), 132.72 (C), 142.57 (C), 150.34 (CH), 155.63 (C), 156.17 (C), 156.61 (C), 167.16 (C). **MS-FAB$^+$**: m/z: 336 [M + 1]. Anal. Calcd for $C_{13}H_{13}N_5O_2S_2$: C, 46.55; H, 3.91; N, 20.88. Found: C, 46.68; H, 3.96; N, 20.89.

3j: **IR [ν, cm^{-1}, KBr]:** 3316 (amine N-H stretching), 3220 (amide N-H stretching), 3078 (aromatic C-H stretching), 2924 (aliphatic C-H asymmetric stretching), 2852 (aliphatic C-H symmetric stretching), 1675 (amide C=O stretching), 1601, 1580, 1460 (C=N and C=C stretching), 1260, 1148, 1055 (C-N stretching). **^{1}H NMR** (400 MHz, DMSO-d_6): δ 1.36 (3H, t, J = 6.7 Hz, benzothiazole O-CH_2-C**H_3**), 3.60 (3H, s, CH_3), 4.07 (2H, q, J = 6.7 Hz, benzothiazole O-C**H_2**-CH_3), 4.21 (2H, s, S-CH_2), 7.03 (1H, d, J = 8.8, 2.4 Hz, benzothiazole H_5), 7.52 (1H, d, J = 2.4 Hz, benzothiazole H_7), 7.62 (1H, d, J = 8.8 Hz, benzothiazole H_4), 8.47 (1H, s, triazole H_5), 12.55 (1H, m, N-H). **^{13}C NMR** (100 MHz, DMSO-d_6): δ 14.66 (CH_3), 21.00 (CH_3), 33.36 (CH_2), 63.62 (CH_2), 105.38 (CH), 115.58 (CH), 121.29 (CH), 132.82 (C), 142.52 (C), 150.39 (CH), 155.83 (C), 155.97 (C), 156.64 (C), 167.26 (C). **MS-FAB$^+$**: m/z: 350 [M + 1]. Anal. Calcd for $C_{14}H_{15}N_5O_2S_2$: C, 48.12; H, 4.33; N, 20.04. Found: C, 48.35; H, 4.31; N, 20.07.

2.2 Microbiology

The study was designed to compare MICs obtained by the CLSI reference M7-A7 broth microdilution method (Koneman et al. 1997; NCCLS 2002). MIC readings were performed twice for each chemical agent. For both the antibacterial and antifungal assays the compounds were dissolved in DMSO. Further dilutions of the compounds and standard drugs in test medium were prepared at the required quantities of 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 μg/mL concentrations with Mueller-Hinton broth and Sabouroud dextrose broth. In order to ensure that the solvent per se had no effect on bacteria or yeast growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilutions used in our experiments and found inactive in culture medium. The observed data on the antibacterial and antifungal activity of the compounds, the control drugs and the test microorganisms are given in Table-2.

RESULTS AND DISCUSSION

At first, 2-chloro-N-(benzothiazol-2-yl)acetamides were synthesized via nucleophilic acyl substitution reaction of 2-aminobenzothiazoles with chloroacetyl chloride in the presence of triethylamine (Bhargava and Ram 1965). The compounds as starting materials which have triazoline-3-thione structure (**2a-b**) were obtained from Aldrich Chemical Co. The desired compounds (**3a-j**) were prepared by reacting 2-chloro-N-(benzothiazol-2-yl)acetamides with appropriate triazoline-3-thiones (**2a-b**) under basic conditions.

Table 2. Antimicrobial Activities of the compounds **3a-j** (mg/mL)

	A	B	C	D	E	F	G	H
3a	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3b	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3c	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3d	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3e	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3f	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3g	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3h	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3i	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
3j	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Reference-1	0.0312	0.0312	0.5	0.0312	0.0156	0.0312	-	-
Reference-2	-	-	-	-	-	-	0.125	0.25

Reference-1: Chloramphenicole, **Reference -2:** Ketoconazole

A: *Escherichia coli* (NRRL B-3008), **B:** *Staphylococcus aureus* (ATCC 6538), **C:** *Pseudomonas aeruginosa* (ATCC 27853), **D:** *Proteus vulgaris* (NRRL B-123), **E:** *Salmonella typhimurium* (ATCC 13311), **F:** Methicillin-resistant *Staphylococcus aureus* (MRSA) (clinic isolate) **G:** *Candida albicans* (NRRL Y-12983) **H:** *Candida parapsilosis* (NRRL Y-12696)

The presence of a base favours the thiol form, which is prone to nucleophilic substitution reactions. These reactions are abbreviated in Scheme.

The compounds which have triazoline-3-thione structure (**2a-b**) may exist in two tautomeric forms, namely thione form and thiol form (Blackman and Polya 1970). In the IR spectra, the absence of a band at 2600-2550 cm^{-1} due to the S-H stretching vibration confirms that the thione form predominates over the thiol form in the solid state.

Acetamide derivatives (**3a-j**) have a strong, characteristic band in the region 1700-1650 cm^{-1} owing to the C=O stretching vibration. The asymmetric and symmetric stretching bands for aliphatic C-H group appear at 2925-2920 cm^{-1} and 2850-2845 cm^{-1} respectively. The aromatic C-H stretching vibration of the compounds (**3a-j**) gives rise to a band at 3100-3000 cm^{-1}.

In the ^{1}H-NMR spectra of the compounds which have triazoline-3-thione structure (**2a-b**), the signals owing to the NH protons, which are showed at 12-13 ppm, are considered as a evidence of the prevalence of the thione form over the thiol form. In the ^{1}H-NMR spectra of acetamide derivatives (**3a-j**), the signal due to the amide proton appears at 12-13 ppm. In their ^{13}C-NMR spectra (**3a-j**), the signal owing to the amide carbon is observed at 167-168 ppm. The mass spectra of compounds showed [M+1] peaks, in agreement with their molecular formula.

MIC's were recorded as the minimum concentration of compound, which inhibits the growth of tested microorganisms. The results indicated that all of the tested compounds were inactive against the test organism.

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