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Synthesis of selected 5-thio-substituted tetrazole derivatives and evaluation of their antibacterial and antifungal activities

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Abstract: Several 5-thio-substituted tetrazole derivatives were efficiently synthesized by a three-step process. The substituted tetrazol-5-thiol, namely, 1-benzyl-1*H*-tetrazole-5-thiol (**2**) was prepared by refluxing commercially available benzyl isothiocyanate (**1**) with sodium azide in water. The second step was the synthesis of 1-benzyl-5-[(3-bromopropyl)thio]-1*H*-tetrazole (**3**) by thioalkylation of tetrazole-5-thiol **2** with 1,3-dibromopropane in tetrahydrofuran. Finally, the 5-thio-substituted tetrazole derivatives **4a**–**i** were prepared by condensation of **3** with the corresponding amine or thiol. The structures of the newly synthesized compounds were characterized by NMR, LC/MS/MS, IR spectral data and elemental analysis. All the synthesized compounds were screened for their antibacterial and antifungal activities.

Keywords: substituted thiol; tetrazole; 1,3-dibromopropane; antibacterial; antifungal.

INTRODUCTION

Tetrazole and its derivatives have attracted interest because of their unique structure and their applications as antihypertensive, anti-allergic, antibiotic and anticonvulsant agents.[1–5] Number of publications and patents on the preparation, properties and applications of tetrazole derivatives is increasing every year with respect to other heterocyclic systems. Development of the tetrazole chemistry has been largely associated with the wide-scale application of these compounds in medicine, biochemistry, agriculture, *etc*.[1–9] The tetrazole functionality plays an

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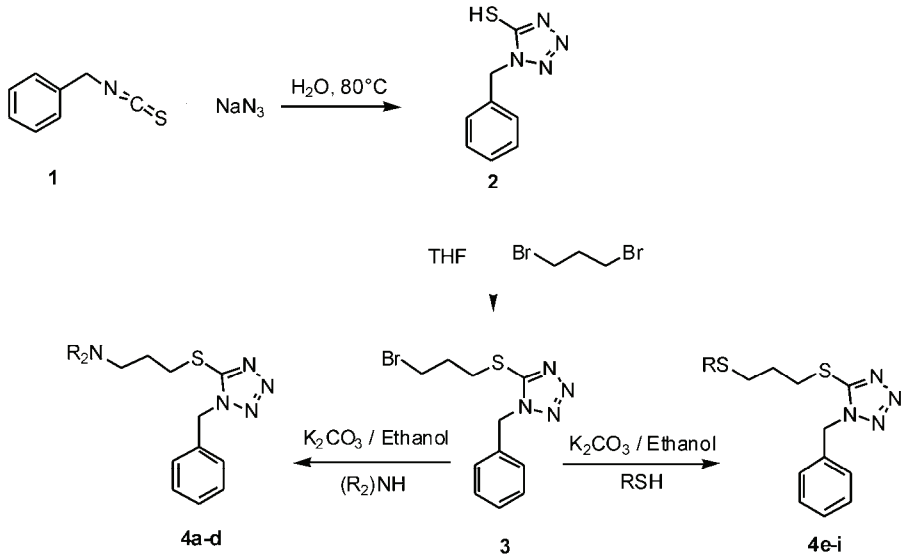
important role in medicinal chemistry, primarily due to its ability to serve as the bioequivalent (bioisostere) of the carboxylic acid group,[10] and also the class of tetrazole compounds has been used both as anticancer and antimicrobial agents.[1–5] In particular, 1-substituted tetrazole and 5-thio-substituted tetrazoles have been used in the synthesis of pharmacologically active drugs.[11–19]

Tetrazoles are quite suitable ligands and can serve as replacement for carboxylic acids not only in medicinal chemistry, but also in supramolecular chemistry. Most importantly, tetrazoles are highly flexible ligands and can adapt easily to different binding modes.[20–23] As there is also a need for new and effective broad-spectrum antifungal and antibacterial agents, it was decided to exploit this interest by ascertaining the molecular features essential for activity and utilizing them to develop a new class of drugs. Prompted by the various biological activities of tetrazole and its 5-thio substituted derivatives, the synthesis of a novel series of 5-thio substituted tetrazole derivatives and a study of their biological activities was envisioned. Thus, the synthesis of the new 5-thio substituted tetrazole derivatives **3** and **4a–i** (Scheme 1 and Table I) and an evaluation of their antibacterial and antifungal properties were the objectives of this study.



Scheme 1. Synthesis scheme for the compounds.

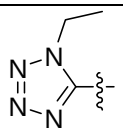
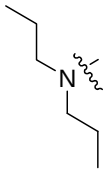
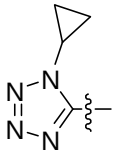
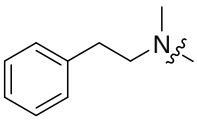
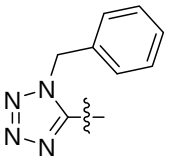
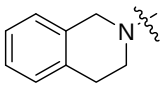
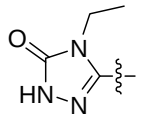
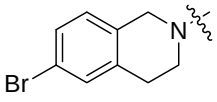
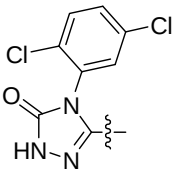
RESULTS AND DISCUSSION

Synthesis

In order to prepare a variety of derivatives of 1-benzyl-5-(propylthio)-1*H*--tetrazole, 1-benzyl-5-[(3-bromopropyl)thio]tetrazole (**3**) was prepared as a precursor. Preparation of 1-benzyl-5-[(3-bromopropyl)thio]tetrazole was accom-

plished as given in Scheme 1. As depicted in Scheme 1, reaction between commercially available benzyl isothiocyanate (**1**) and sodium azide in water provided 1-benzyl-1*H*-tetrazole-5-thiol[24] (**2**) in good yield. The isolated compound **2** was treated with 1,3-dibromopropane in tetrahydrofuran to give an intermediate, 1-benzyl-5-[(3-bromopropyl)thio]-1*H*-tetrazole (**3**). The synthon **3** is a new compound and reported here for the first time. Compound **3** was treated with corresponding amines or thiols to afford the 5-thio-substituted tetrazole derivatives **4a**–**i**.

TABLE I. Structure of the substituent (R) in the compounds **4a**–**i**

Compound	$(R_2)N$	Compound	R
3	Br	**4e**	
4a		**4f**	
4b		**4g**	
4c		**4h**	
4d		**4i**	

Characterization

The structures of the synthesized compounds were elucidated by ^{1}H-NMR, ^{13}C-NMR, LC–MS and IR spectroscopy, and elemental analysis, the results of which are given below.

*1-Benzyl-5-[(3-bromopropyl)thio]-1*H*-tetrazole (**3**).* Anal. Calcd. for $C_{11}H_{14}BrN_4S$: C, 42.14; H, 4.47; N, 17.88 %. Found: C, 42.08; H, 4.46; N,

17.85 %. IR (KBr, cm^{-1}): 2964 (C–H), 1496 (N=N), 1453 (C=C), 1389 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 2.29–2.36 (2H, *qn*), 3.42–3.49 (4H, *m*), 5.42 (2H, *s*), 7.27–7.44 (5H, *m*). ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 31.2, 31.5, 153.4, 51.0, 128.1, 129.0, 129.1, 132.7. MS Calcd. for $C_{11}H_{14}BrN_4S$: 313.22; Found: M^++1, 312.9, 314. 9. MS/MS (*m*/*z*): 262.2, 233.6, 178.2, 102.0, 90.7.

*3-(1-Benzyl-1*H*-tetrazol-5-ylthio)*-N,N-*dipropylpropan-1-amine* ***(4a)***. Yield: 62 %; Anal. Calcd. for $C_{17}H_{27}N_5S$: C, 61.17; H, 8.10; N, 20.99 %. Found: C, 61.08; H, 8.08; N, 21.03 %. IR (KBr, cm^{-1}): 2957 (C–H), 1497 (N=N), 1455 (C=C), 1388 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 0.84–0.88 (6H, *t*), 1.36–1.46 (4H, *m*), 1.85–1.91 (2H, *qn*), 2.30–2.34 (4H, *t*), 2.46–2.50 (2H, *t*), 3.33––3.37 (2H, *t*), 5.41 (2H, *s*), 7.27–7.39 (5H, *m*). ^{13}C-NMR (100 MHz, $CDCl_3$, δ / / ppm): 11.9, 20.2, 26.9, 31.5, 56.0, 52.3, 128.0, 128.8, 129.0, 133.0, 154.3. MS calculated for $C_{17}H_{27}N_5S$: 333.49; Found: M^++1, 334.0. MS/MS (*m*/*z*): 233.4, 174.2, 132.3, 104.2, 90.1.

*3-(1-Benzyl-1*H*-tetrazol-5-ylthio)*-N-*methyl*-N-*phenethylpropan-1-amine* ***(4b)***. Yield: 78 %. Anal. Calcd. for $C_{20}H_{25}N_5S$: C, 65.30; H, 6.80; N, 19.05 %. Found: C, 65.37; H, 6.79; N, 19.09 %. IR (KBr, cm^{-1}): 2947 (C–H), 1496 (N=N), 1453 (C=C), 1389 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 1.89––1.94 (2H, *qn*), 2.26 (3H, *s*), 2.45–2.49 (2H, *t*), 2.56–2.60 (2H, *t*), 2.71–2.76 (2H, *t*), 3.26–3.30 (2H, *t*), 5.39 (2H, *s*), 7.17–7.36 (10H, *m*). ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 26.8, 31.2, 33.7, 41.9, 50.8, 55.5, 59.4, 125.9, 128.1, 128.3, 128.7, 128.9, 129.0, 132.9, 140.4, 154.2. MS calculated for $C_{20}H_{25}N_5S$: 367.51; Found: M^++1, 368.1. MS/MS (*m*/*z*): 368.1, 339.8, 233.2, 208.0, 176.1, 105.2, 90.9.

*2-[3-(1-Benzyl-1*H*-tetrazol-5-ylthio)propyl]-1,2,3,4-tetrahydroisoquinoline* ***(4c)***. Yield: 78 %; Anal. Calcd. for $C_{20}H_{23}N_5S$: C, 65.66; H, 6.29; N, 19.15 %. Found: C, 65.60; H, 6.30; N, 19.16 %. IR (KBr, cm^{-1}): 2922 (C–H), 1497 (N=N), 1453 (C=C), 1388 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 2.0––2.08 (2H, *qn*), 2.57–2.60 (2H, *t*), 2.67–2.70 (2H, *t*), 2.86–2.88 (2H, *t*), 3.36–3.39 (2H, *t*), 3.59 (2H, *s*), 5.37 (2H, *s*), 6.99–7.33 (9H, m). ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 26.7, 50.9, 31.5, 29.1, 50.8, 56.0, 56.3, 128.1, 129.0, 125.6, 126.1, 126.5, 128.6, 128.8, 133.0, 134.2, 134.6, 154.2. MS calculated for $C_{20}H_{23}N_5S$: 365.50; Found: M^++1, 366.1; M^++K adduct: 404.2. MS/MS (*m*/*z*): 338.0, 233.1, 206.1, 101.9, 91.0.

*2-[3-(1-Benzyl-1*H*-tetrazol-5-ylthio)propyl]-6-bromo-1,2,3,4-tetrahydroiso-quinoline* ***(4d)***. Yield: 72 %; Anal. Calcd. for $C_{20}H_{22}BrN_5S$: C, 54.01; H, 4.95; N, 15.75 %. Found: C, 54.10; H, 4.95; N, 15.72 %. IR (KBr, cm^{-1}) 2924 (C–H), 1482 (N=N), 1453 (C=C), 1388 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 2.00–2.06 (2H, *qn*), 2.55–2.58 (2H, *t*), 2.65–2.68 (2H, *t*), 2.77–2.80 (2H, *t*), 3.35––3.38 (2H, *t*), 3.53 (2H, *s*), 5.37 (2H, *s*), 6.93–7.33 (8H, *m*). ^{13}C-NMR (100

MHz, $CDCl_3$, δ / ppm): 26.6, 50.1, 28.5, 45.9, 31.4, 55.4, 56.0, 119.0, 128.1, 129.0, 128.8, 129.1, 129.3, 132.9, 133.3, 136.9, 154.2. MS calculated for $C_{20}H_{22}BrN_5S$: 444.39; Found: M^++1, 444.1. MS/MS (*m*/*z*): 415.9, 284.0, 102.2, 91.0.

*1-Benzyl-5-[3-(1-ethyl-1*H*-tetrazol-5-ylthio)propylthio]-1*H*-tetrazole* ***(4e)***. Yield: 68 %; Anal. Calcd. for $C_{14}H_{18}N_8S_2$: C, 46.35; H, 4.97; N, 30.90 %. Found: C, 46.26; H, 4.96; N, 30.82 %. IR (KBr, cm^{-1}): 2983 (C–H), 1496 (N=N), 1433 (C=C), 1390 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 1.48––1.52 (3H, *t*), 2.27–2.34 (2H, *qn*), 3.40–3.44 (4H, *q*), 4.24–4.29 (2H, *q*), 5.44 (2H, *s*), 7.26–7.38 (5H, *m*). ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 14.2, 28.8, 31.3, 31.6, 42.6, 51.0, 128.0, 128.9, 129.0, 132.8, 152.8, 153.5. MS calculated for $C_{14}H_{18}N_8S_2$: 362.48; Found: M^++1, 363.1; M^++Na adduct: 385.2; M^++K adduct: 401.0. MS/MS (*m*/*z*): 335.0, 306.2, 265.0, 233.0, 105.9, 91.0.

*1-Benzyl-5-[3-(1-cyclopropyl-1*H*-tetrazol-5-ylthio)propylthio]-1*H*-tetrazole* ***(4f)***. Yield: 70 %; Anal. Calcd. for $C_{15}H_{18}N_8S_2$: C, 48.07; H, 4.81; N, 29.91 %. Found: C, 48.13; H, 4.80; N, 29.89 %. IR (KBr, cm^{-1}): 2938 (C–H), 1496 (N=N), 1454 (C=C), 1392 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 1.22––1.29 (4H, *m*), 2.29–2.36 (2H, *qn*), 3.35–3.37 (1H, *m*), 3.41–3.45 (4H, *q*), 5.44 (2H, *s*), 7.26–7.39 (5H, *m*). ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 6.8, 28.8, 27.8, 30.8, 31.7, 51.0, 128.1, 128.9, 129.0, 132.8, 153.5, 155.5. MS calculated for $C_{15}H_{18}N_8S_2$: 374.49; Found: M^++1, 375.3; M^++Na adduct: 397.0; M^++K adduct: 413.1. MS/MS (*m*/*z*): 265.4, 233.4, 183.3, 102.0, 91.2.

*5,5'-[1,3-Propanediylbis(thio)]bis(1-benzyl-1*H*-tetrazole)* ***(4g)***. Yield: 41 %; Anal. Calcd. for $C_{19}H_{20}N_8S_2$: C, 53.70; H, 4.71; N, 26.38 %. Found: C, 53.78; H, 4.72; N, 26.43 %. IR (KBr, cm^{-1}): 2942 (C–H), 1496 (N=N), 1453 (C=C), 1389 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 2.21–2.28 (2H, *qn*), 3.33––3.37 (4H, *t*), 5.41 (4H, *s*), 7.25–7.37 (10H, *m*). ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 28.7, 31.5, 50.8, 128.0, 128.8, 128.9, 133.0, 153.5. MS calculated for $C_{19}H_{20}N_8S_2$: 424.55; Found: M^++1, 425.4; M^++Na adduct: 447.1. MS/MS (*m*/*z*): 282.9, 265.2, 233.0, 130.9, 102.0, 90.9.

*5-[3-(1-Benzyl-1*H*-tetrazol-5-ylthio)propylthio]-4-ethyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one* ***(4h)***. Yield: 48 %; Anal. Calcd. for $C_{15}H_{19}N_7OS_2$: C, 47.68; H, 5.03; N, 25.96 %. Found: C, 47.78; H, 5.02; N, 25.99 %. IR (KBr, cm^{-1}): 1751 (C=O), 2936 (C–H), 3100 (N–H), 1523 (N=N), 1451 (C=C), 1389 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 1.27–1.30 (3H, *t*), 2.21–2.28 (2H, *qn*), 3.15–3.19 (2H, *t*), 3.42–3.45 (2H, *t*), 3.66–3.72 (2H, *q*), 5.45 (2H, *s*), 7.29–7.37 (5H, *m*), 11.34 (1H, *bs*). ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 14.2, 28.7, 29.8, 31.7, 36.6, 51.0, 128.0, 128.9, 129.0, 132.8, 143.4, 153.5, 155.9. MS calculated for $C_{15}H_{19}N_7OS_2$: 377.49; Found: M^++1, 378.4. MS/MS (*m*/*z*): 308.0, 233.3, 218.1, 186.0, 101.9, 91.0.

*5-[3-(1-Benzyl-1*H*-tetrazol-5-ylthio)propylthio]-4-(2,5-dichlorophenyl)-2,4--dihydro-3*H*-1,2,4-triazol-3-one (**4i**)*. Yield: 39 %. Anal. Calcd. for $C_{19}H_{17}Cl_2N_7OS_2$: C, 46.11; H, 3.44; N, 19.82 %. Found: C, 46.16; H, 3.43; N, 19.76 %. IR (KBr, cm^{-1}): 1718 (C=O), 2900 (C–H), 1519 (N=N), 1478 (C=C), 1425 (C=N). ^{1}H-NMR (400 MHz, $CDCl_3$, δ / ppm): 2.15–2.20 (2H, *qn*), 3.05–3.08 (2H, *t*), 3.33––3.38 (2H, *t*), 5.40 (2H, *s*), 7.24–7.51 (7H, *m*), 10.94 (1H, *s*). ^{13}C-NMR (100 MHz, $CDCl_3$, δ / ppm): 28.5, 29.8, 31.6, 51.0, 128.1, 128.9, 129.0, 130.4, 130.7, 131.5, 131.6, 131.8, 132.8, 133.5, 143.7, 153.5, 154.6. MS calculated for $C_{19}H_{17}Cl_2N_7OS_2$: 494.42; Found: M^++1, 493.8. MS/MS (*m*/*z*): 334.1, 309.1, 302.0, 288.0, 265.6, 233.4, 165.3, 102.2, 91.2.

In the IR spectra, the bands due to –N=N– and C=N group, present in all compounds, were observed at about 1500 and 1388 cm^{-1}, respectively. The bands at about 1244 and 985 cm^{-1} are characteristic for the tetrazole ring system.[25] In the ^{1}H-NMR spectra, the 1-substituted benzylic protons appeared as singlet at about δ 5.4 ppm in all the derivatives. The other methylene protons appeared as quintets and triplets at about δ 2.2 to 2.3 ppm and 3.3 to 3.5 ppm, respectively. The aromatic protons were observed at about δ 6.9 to 7.4 ppm. In the ^{13}C-NMR spectra of all the synthesized compounds, tetrazole carbon and aromatic carbon peaks were observed at δ 153.5 and 128.0 to 129.5 ppm, respectively. In the mass spectra, an appropriate molecular ion peak (M^++1) was obtained for all the derivatives from ESI–MS.

Antibacterial activity

The results of the antibacterial studies against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* bacterial strains are given in Table II and compared with the standard ampicillin drug. Interestingly, out of eleven compounds, nine compounds were found to have

TABLE II. Antibacterial activities of the compounds **2**, **3** and **4a–i** (zone of inhibition in mm; minimum inhibitory concentration (*MIC*), in mg mL^{-1}, is given in parenthesis)

Compound	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*
2	15 (6.25)	<10 (50)	<10 (50)	<10 (50)
3	20 (6.25)	18 (6.25)	19 (6.25)	19 (6.25)
4a	17 (6.25)	18 (6.25)	<10 (50)	<10 (50)
4b	18 (6.25)	17 (6.25)	<10 (50)	<10 (50)
4c	17 (6.25)	18 (6.25)	<10 (50)	<10 (50)
4d	19 (6.25)	19 (6.25)	<10 (50)	<10 (50)
4e	20 (6.25)	19 (6.25)	<10 (50)	<10 (50)
4f	17 (6.25)	17 (6.25)	<10 (50)	<10 (50)
4g	20 (6.25)	19 (6.25)	19 (6.25)	20 (6.25)
4h	<10 (50)	<10 (50)	<10 (50)	<10 (50)
4i	19 (6.25)	17 (6.25)	<10 (50)	<10 (50)
Ampicillin	22 (6.25)	23 (6.25)	20 (6.25)	22 (6.25)

good antibacterial activity. Among these compounds **3** and **4g** were most active against the four bacterial organisms. Compounds **4a**–**f** and **4i** showed good growth inhibition towards *S. aureus* and *E. coli*, and pronounced growth inhibition for *P. aeruginosa* and *K. pneumoniae*. The remaining compounds, **2** and **4h**, were found to be less active against the four bacterial organisms.

Antifungal activity

The results of the antifungal studies against *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium marneffei* and *Trichophyton mentagrophytes* are given in Table III and compared with the standard itraconazole drug. It was observed that most of the compounds exhibited good antifungal activity. Compounds **2** and **4h** were less active against all the tested organisms. Compounds **4a**–**f** and **4i** showed good antifungal activity against *P. marneffei* and *T. mentagrophytes*, and pronounced antifungal activity towards the other two fungal organisms. On the other hand, compounds **3** and **4g** showed the highest antifungal activity against all four fungal strains, namely *A. flavus*, *A. fumigatus*, *P. marneffei* and *T. menta-grophytes*.

TABLE III. Antifungal activities of the compounds **2**, **3** and **4a–i** (zone of inhibition in mm, *MIC*, in mg mL^{-1}, is given in parenthesis)

Compound	*T. mentagrophytes*	*P. marneffei*	*A. flavus*	*A. fumigatus*
2	13 (6.25)	<10 (50)	<10 (50)	<10 (50)
3	19 (6.25)	17 (6.25)	20 (6.25)	19 (6.25)
4a	19 (6.25)	18 (6.25)	<10 (50)	<10 (50)
4b	18 (6.25)	19 (6.25)	<10 (50)	<10 (50)
4c	18 (6.25)	17 (6.25)	<10 (50)	<10 (50)
4d	19 (6.25)	18 (6.25)	<10 (50)	<10 (50)
4e	19(6.25)	18 (6.25)	<10 (50)	<10 (50)
4f	18 (6.25)	19 (6.25)	<10 (50)	<10 (50)
4g	20 (6.25)	18 (6.25)	19 (6.25)	20 (6.25)
4h	<10 (50)	<10 (50)	<10 (50)	<10 (50)
4i	19 (6.25)	17 (6.25)	<10 (50)	<10 (50)
Itraconazole	21(6.25)	20 (6.25)	21 (6.25)	19 (6.25)

EXPERIMENTAL

The ^{1}H-NMR, ^{13}C-NMR and DEPT experiments were performed on an Oxford AS 400 NMR instrument (Varian, City, USA) with a dual broad band. The ^{1}H-NMR chemical shift values are reported on the δ scale in ppm relative to TMS (δ = 0 ppm) and the ^{13}C-NMR chemical shifts values are reported relative to $CDCl_3$ (δ = 72.5 ppm). The IR spectra were recorded on a Perkin Elmer spectrum 100 FT-IR model. Column chromatography was performed with silica gel 60–120 mesh (Merck, Mumbai, India.). All the compounds were routinely checked for completion of the reaction on silica gel 60 F254 TLC plates and their spots were visualized by exposure to a UV lamp, iodine vapor or $KMnO_4$ reagents. The liquid chromatography part of the LC–MS system consisted of an Agilent-1100 series quaternary gradient pump with a degasser, an auto sampler and a column oven. The MS/MS part of the sys-

tem contained an API-2000 system (Sciex, Applied Bio-Systems, Canada). The yields are reported as isolated yield after purification of the compounds.

*Procedure for the preparation of 1-benzyl-5-[(3-bromopropyl)thio]-1*H*-tetrazole (**3**)*

To a solution of 1,3-dibromopropane (10 g, 50 mmol) in tetrahydrofuran (200 mL), 1--benzyl-1*H*-tetrazole-5-thiol (**2**) (1.92 g, 10 mM) was added portionwise at 25–30 °C and then stirred for 3 h at the same temperature. The reaction was monitored by TLC. After completion of reaction, the reaction mixture was concentrated to dryness and the crude product was purified by column chromatography (eluent 35 % ethyl acetate–hexane). Product **3** was obtained as light brown colored gummy mass (52 % yield).

*General procedure for the preparation of derivatives **4a–i***

To a mixture of 1-benzyl-5-[(3-bromopropyl)thio]-1*H*-tetrazole (**3**) (10 mmol) and anhydrous powdered K_2CO_3 (20 mmol) in ethanol (10 mL), the corresponding amine or thiol (1.2 equivalent) was added at 25 °C and stirred at this temperature for 2–3 h. The reaction was monitored by TLC. After completion of the reaction; the reaction mixture was concentrated to dryness. The residue was dissolved in dichloromethane (20 mL) and washed with water. The organic layer was concentrated under reduced pressure to give the crude product. The final product was isolated by column purification. The column was started at 10 % ethyl acetate with petroleum ether and slowly increased to 60 % ethyl acetate. Finally, the compound was isolated at 25 to 30 % ethyl acetate in petroleum ether.

Antibacterial activity

The tetrazole derivatives (**2**, **3** and **4a–i**) were investigated for their inhibition of growth against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-27853) and *Klebsiella pneumoniae* (recultured) bacterial strains by the disc diffusion method.[26-31] Batches of 100 discs (Whatman filter paper No. 1, 6 mm diameter) were each dispensed to a screw capped bottle and sterilized by dry heat at 140 °C for 1 h. Solutions of the test compounds were prepared at different concentrations in dimethylformamide (DMF). 1mL containing 100 times the amount of prepared solution in each disc was added to each bottle, which contained 100 discs. The disc of each concentration was placed in triplicate in a nutrient agar medium separately seeded with fresh bacteria. The incubation was realized at 37 °C for 24 h. Solvent and growth controls were kept separate; the zones of inhibition and minimum inhibitory concentration (*MIC*) were measured.

Antifungal activity

The newly synthesized compounds were also investigated for their antifungal activity against four fungal strains, namely, *Aspergillus flavus* (NCIM No.524), *Aspergillus fumigatus* (NCIM No.902), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured). Sabouraud agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in sterile water (100 mL) and the pH was adjusted to 5.7. Normal saline was used to make a suspension of the spores of the fungal strain for seeding. A loopful of particular fungal strain was transferred to 3 mL of saline to obtain a suspension of the corresponding species. Agar media (20 mL) was poured into each petri dish. Excess of the suspension was decanted and the plates were dried by placing them in an incubator at 37 °C for 1 h. Using an agar punch, wells (8 mm diameter) were made on these seeded agar plates and from 6.25 to 50 μg mL^{-1} of the test compounds in DMSO was added into each well labeled disc. Controls were run using DMSO at the same concentration as used with the test compounds. The petri dishes were pre-

pared in triplicate and maintained at 37 °C for 3 to 4 days. The antifungal activity was determined by measuring the diameter of the inhibition zone.[32,33]

CONCLUSIONS

In conclusion, a series of new 5-thio-substituted tetrazole derivatives were successfully synthesized. The antimicrobial screening suggests that all the synthesized compounds showed moderate to good activity against the tested organisms. Among the newly synthesized compounds, **4g** and **3** showed the most promising antibacterial and antifungal activities. Hence, the fact that the compounds prepared in this study are chemically unrelated to the current medication, suggests that further work with similar types of analogues is clearly warranted.

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ИЗВОД

СИНТЕЗА ОДАБРАНИХ 5-ТИО СУПСТИТУИСАНИХ ДЕРИВАТА ТЕТРАЗОЛА И ОДРЕЂИВАЊЕ ЊИХОВЕ АНТИБАКТЕРИЈСКЕ И АНТИФУНГАЛНЕ АКТИВНОСТИ

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Извршена је синтеза нових деривата 5-тио-тетразола. Супституисани тетразол-5-тиол, 1-бензил-1*H*-тетразол-5-тиол (**2**) добијен је загревањем на температури кључања комерцијално доступног бензил-изотиоцијаната (**1**) са натријум-азидом у води. У другом реакционом кораку добијен је 1-бензил-5-[(3-бромпропил)тио]-1*H*-тетразол (**3**) тиоалкиловањем тетразол-5-тиола (**2**) са 1,3-дибромпропаном у тетрахидрофурану. Коначно, деривати 5-тио-тетразола **4a**–**i** добијени су кондензациојом (**3**) са одговарајућим аминима или тиолима. Структуре добијених једињења одређене су NMR, LC/MS/MS и IC спектроскопијом и микроанализом. Свим синтетисаним једињењима испитана је антибактеријска и антифунгална активност.

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