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Microwave-assisted synthesis of *N*-alkylated benzotriazole derivatives: Antimicrobial studies

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Abstract—Synthesis and characterization of *N*-alkylated benzotriazole derivatives **2(a–g)** bearing pharmaceutically important bioactive substituents and their antimicrobial studies in vitro are described. The syntheses of the compounds were achieved by *N*-alkylation of the benzotriazole with different bioactive alkyl halides in presence of powdered K_2CO_3 in DMF solution and by microwave irradiation method with good yield compared to conventional method. The crystal structure analysis shows that compound 4′-benzotriazol-1-yl-methyl-biphenyl-2-carbonitrile **2a** crystallizes in the space group P1 with cell parameters $a = 8.526$ (3) Å, $b = 12.706$ (3) Å, $c = 7.966$ (2) Å, $\alpha = 100.89$ (2)°, $\beta = 101.63$ (3)°, $\gamma = 102.20$(2)°, volume = 801.7(4) Å^3, $Z = 2$ and the final *R* factor is 0.0559 for 6130 reflections with 218 parameters and zero restraint. This structure exhibits intermolecular hydrogen bonding. Compounds **2e, 2a** showed significant antimicrobial activity.

Benzotriazole can be a good leaving group, acts as an ambient anion director, an electron donor, radical or carbanion precursor. Benzotriazole is easy to introduce into molecules by a variety of condensations, additions, and benzotriazolyl-alkylation reactions.[1–3] Earlier, we have reported the synthesis of stable nitrenium ions using benzotriazole as a synthon.[4] Benzotriazole derivatives are of biological, chemical, and industrial importance. These derivatives exhibit a good degree of analgesic, anti-inflammatory, diuretic, antiviral, and antihypertensive activities.[5–7] Microwave-assisted reactions have become an established tool in organic synthesis.[8] In continuation of our studies in synthesizing various bioactive heterocycles,[9–12] we herein report the microwave-mediated synthesis, characterization, and crystal structure analysis of some newer *N*-alkylated benzotriazole derivatives, which bear bioactive key intermediates and their efficacy as antimicrobials.

The synthesis of the title compounds was done by *N*-alkylation of benzotriazole with different bioactive alkyl halides such as 4′-bromomethyl-biphenyl-2-carbonitrile, 2-bromo-4,5,dimethoxy benzyl bromide, 5-methyl-6-chloro-methyl-benzo[1,3] dioxole, etc., in the presence of powdered K_2CO_3 in DMF solution (Scheme 1). The compounds **2(a–g)** were also obtained from microwave irradiation for a short period of time (30–40 s) at low power setting in DMF solution. In comparison to the conventional (thermal) heating method, microwave heating offers more advantages such as reduced reaction time (30–40 s), low cost, simplicity in processing, reduced pollution, and high yield. The *N*-alkylated benzotriazole derivatives **2(a–g)** were characterized by ^{1}H NMR, C, H, N analysis and X-ray crystal structure analysis (Table 1). The ^{1}H NMR spectrum of the compounds, showing a singlet for two protons in the range of 5.8–6.8 ppm, is assigned to the N-CH_2- group of benzotriazole protons. The aromatic protons resonate in the region of 7.0–7.8.[17]

Crystal structure of the compound 4′benzotriazol-1-yl-methyl-biphenyl-2-carbonitrile **2a** was determined by the X-ray diffraction method.[18] The details are listed in Table 2. The ORTEP of the compound **2a** at 50% probability is given in Figure 1. The packing of the molecule

Keywords: Benzotriazole; N-alkylation; Antimicrobials; Crystal structure.
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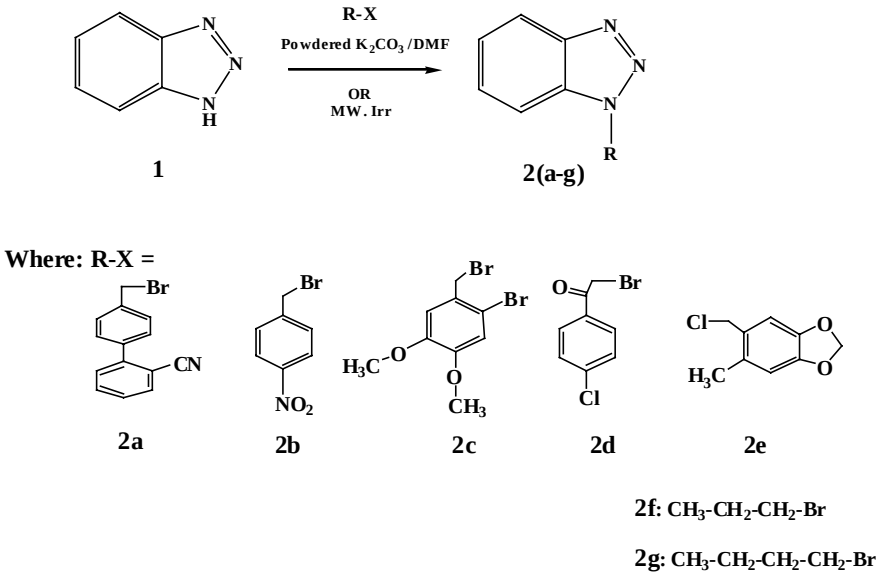
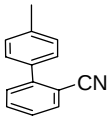
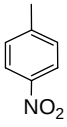
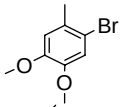
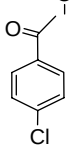
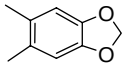


Scheme 1.

Table 1. Physical data of the compounds **2(a–g)**

Compound	R	R_f value	Eluent	Yield (%)		mp (°C)
				K_2CO_3/DMF	Microwave	
2a		0.83	Benzene:ethyl acetate 9:1	82	86	145–147
2b		0.74	Benzene:ethyl acetate 9:1	79	85	109–112
2c		0.78	Benzene:ethyl acetate 9:1	86	92	94–96
2d		0.80	Benzene:ethyl acetate 9:1	69	79	135–137
2e		0.65	Benzene:ethyl acetate 9:1	82	95	85–88
2f	CH_3-CH_2-CH_2-	0.75	Benzene:ethyl acetate 9:1	70	86	Oily
2g	CH_3-CH_2-CH_2-CH_2-	0.82	Benzene:ethyl acetate 9:1	65	76	Oily

along the *a* axis is presented in Figure 3A. The interhydrogen bonding of the molecule **2a** is shown in Figure 3B. The compound exhibits intermolecular hydrogen bonding between N1——C23-H23 with bond length 3.461(3) Å.

In view of synthesizing new antimicrobials, we have synthesized newer benzotriazole derivatives **2(a–g)** and evaluated their efficacy as antimicrobials in vitro by the disk diffusion method[16,19] against different strains. Tests were performed in triplicate and the results are reported as means of at least three determinations. Inhibitory activity of the compounds against both bacterial and fungal strains was observed in the following order **2e > 2a > 2c > 2g** as shown in Tables 3 and 4. The presence of benzo[*d*][1,3]dioxole group

Table 2. Crystallographic data of the **2a**

Empirical formula	C_{20} H_{14} N_4
Formula weight	310.35
Temperature	293(2) K
Wavelength	0.71069 Å
Crystal system	Triclinic
Space group	P 1
Cell dimensions	
a	8.526(3) Å
b	12.706(3) Å
c	7.966(2) Å
α	100.89(2)°
β	101.63(3)°
γ	102.20(2)°
Volume	801.7(4) A^3
Z	2
Density (calculated)	1.286 Mg/m^3
Absorption coefficient	0.079 mm^{-1}
Crystal size	$0.2 \times 0.2 \times 0.25$ mm
Theta range for data collection	2.53–32.50°
Reflections collected	6130
Independent reflections	5795
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	5795/0/218
Final *R* indices [$I > 2\sigma$ (*I*)]	$R1 = 0.0559$, $\omega R2 = 0.1713$
R indices (all data)	$R1 = 0.1211$, $\omega R2 = 0.2265$
Extinction coefficient	0.007(6)
Largest diff. peak and hole	0.263 and -0.263 e A^{-3}
Measurement	Rigaku AFC7S
Program system	teXsan
Structure determination	SHELXS-97
Structure refinement	SHELXL-97

in **2e** may be responsible for the significant inhibitory activity and in compound **2a**, the presence of bioactive 2′-cyano-biphenyl ring would enhance the inhibitory activity of **2a** than the standard drugs at the concentration tested, indicating that compounds **2e** and **2a** are significant antimicrobials in vitro under the benzotriazole class of compounds (Fig. 2). Compounds **2c** and **2g** bearing 2-bromo-4, 5-dimethoxy-benzyl, and 1-butyl groups, respectively, exhibited equipotency compared to the standard drugs and other compounds were found to be not effective against any of the strains tested.

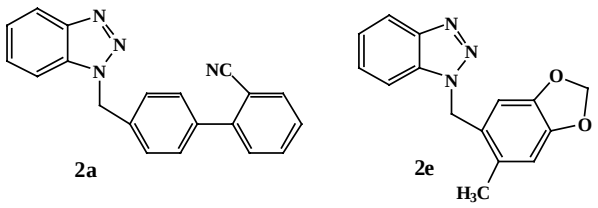


Figure 2. Structures of potent molecules.

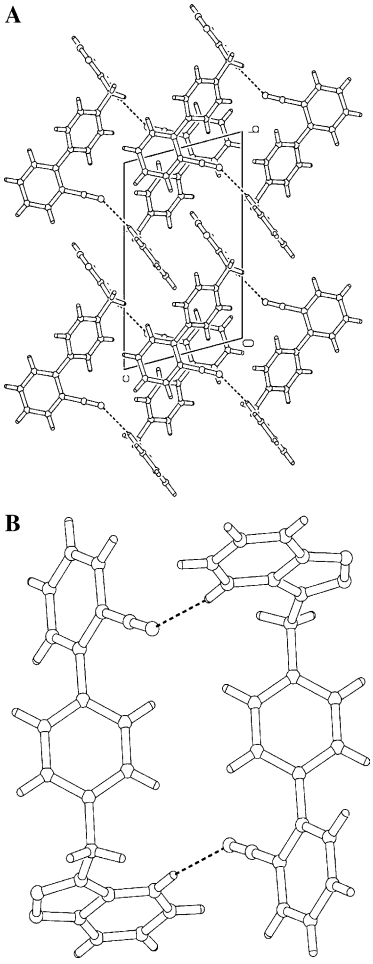


Figure 3. (A) Packing of the molecule **2a** down *a* axis. (B) Hydrogen bonds in the molecule **2a**.

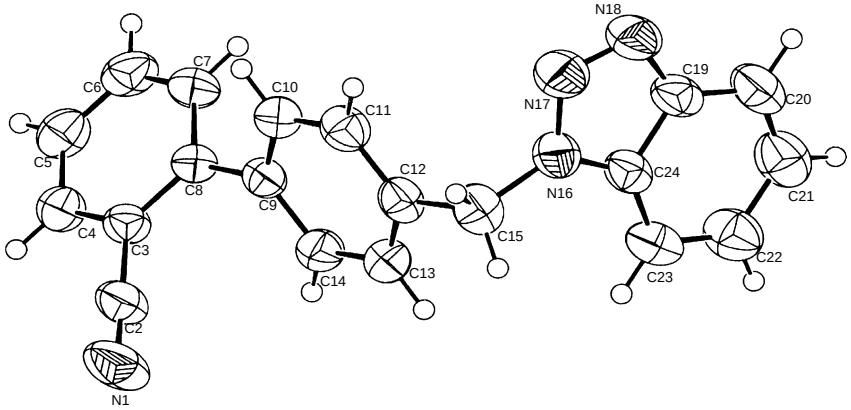


Figure 1. ORTEP diagram of the molecule **2a** at 50% probability. The selected bond lengths are: C8–C9: 1.487 Å, C12–C15: 1.513 Å, C15–N16: 1.467 Å, N16–N17: 1.353 Å, N17–N18: 1.308 Å, C19–N20: 1.423 Å, and C2–N1: 1.138 Å. The selected bond angles are: C8–C9–C10: 120.19°, C7–C8–C9: 120.42°, C12–C15–N16: 111.58°, N17–N18–C19: 108.04°, and N18–N17–N16: 108.75°.

Table 3. Inhibitory zone (diameter) mm of compounds against tested bacterial strains by the disk diffusion method

Compound	Inhibitory zone (diameter) mm[a]				
	Bacillus subtilis	*Escherichia coli*	*Pseudomonas fluorescens*	*Xanthomonas campestris* pvs.	*Xanthomonas oryzae*
2a	16 ± 0.65	18 ± 0.78	21 ± 0.82	16 ± 0.71	15 ± 0.68
2b	6 ± 0.21	7 ± 0.28	8 ± 0.32	4 ± 0.11	3 ± 0.12
2c	12 ± 0.48	15 ± 0.68	18 ± 0.79	12 ± 0.49	11 ± 0.47
2d	6 ± 0.28	5 ± 0.19	3 ± 0.11	4 ± 0.14	2 ± 0.06
2e	18 ± 0.75	20 ± 0.8	22 ± 0.81	18 ± 0.81	19 ± 0.81
2f	6 ± 0.21	4 ± 0.16	1 ± 0.03	3 ± 0.12	2 ± 0.08
2g	13 ± 0.59	14 ± 0.61	19 ± 0.82	12 ± 0.51	12 ± 0.41
Streptomycin	12 ± 0.51	14 ± 0.64	18 ± 0.78	—	—
Tetracycline	—	—	—	12 ± 0.54	11 ± 0.49
DMSO	0.8 ± 0.03	0.6 ± 0.01	0.9 ± 0.031	0.5 ± 0.02	0.7 ± 0.025

Streptomycin sulfate (10 μg/disk); Tetracycline (10 μg/disk) were used as positive reference, compounds (25 μg/disk).
[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.

Table 4. Inhibitory zone (diameter) mm of compounds against tested fungal strains by the disk diffusion method

Compound	Inhibitory zone (diameter) mm[a]				
	Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporum*	*Trichoderma* species	*Fusarium monaliforme*
2a	14 ± 0.61	17 ± 0.79	20 ± 0.8	18 ± 0.72	17 ± 0.79
2b	5 ± 0.21	7 ± 0.31	9 ± 0.39	11 ± 0.49	8 ± 0.38
2c	9 ± 0.41	10 ± 0.41	14 ± 0.61	16 ± 0.72	13 ± 0.59
2d	4 ± 0.17	5 ± 0.19	6 ± 0.21	2 ± 0.81	3 ± 0.12
2e	16 ± 0.62	19 ± 0.90	22 ± 0.9	20 ± 0.91	18 ± 0.8
2f	5 ± 0.18	4 ± 0.11	6 ± 0.21	1 ± 0.03	4 ± 0.1
2g	8 ± 0.31	11 ± 0.41	14 ± 0.58	15 ± 0.68	12 ± 0.48
Nystatin	8 ± 0.34	10 ± 0.31	14 ± 0.52	16 ± 0.72	12 ± 0.50
DMSO	0.8 ± 0.03	0.6 ± 0.02	0.5 ± 0.021	0.3 ± 0.012	0.9 ± 0.03

Nystatin (10 μg/disk) was used as positive reference, compounds (25 μg/disk).
[a] Values are means of three determinations, the ranges of which are less than 5% of the mean in all cases.

In conclusion, compounds **2a** 4′benzotriazol-1-yl-methyl-biphenyl-2-carbonitrile and 1-((5-methylbenzo[1,3]-dioxol-6-yl)methyl)-1*H*-benzotriazole **2e** are potent antimicrobials of this class of compounds. This inhibitory activity might be attributed to the presence of bulky hydrophobic groups present (cyano-biphenyl in **2a** and benzo dioxole in **2e**).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.10.084. The full crystallographic details have been deposited at Cambridge Crystallography Data Center (CCDC No. 277414).

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17. Silica gel GF-254 was used for thin layer chromatography. Melting points were recorded on a SEALCO-605 melting point apparatus and are uncorrected. IR spectra were recorded on a FT-IR 8000 spectrometer. ^{1}H NMR spectra were recorded on Bruker AMX-400 MHz using $CDCl_3$ as solvent with TMS as internal standard. Elemental analysis was obtained on a Vario-EL instrument. *General procedure for the synthesis of N-alkylated benzotriazole derivatives*: Equimolar mixtures of benzotriazole and alkyl halides were dissolved in DMF solution and 3 equivalents of powdered potassium carbonate were added. The reaction mass was stirred at room temperature overnight until the reaction was completed, which was monitored by TLC. After completion of the TLC, the reaction mass was poured into 10 volumes of water, the compounds were extracted in ethyl acetate (6 volumes × 3), and the combined organic layer was washed with water and distilled completely. The pure compounds were obtained by adding 4 volumes of *n*-hexane, cooling for 2 h at 10–15 °C, and filtering the mass at the same temperature. *Microwave irradiation method*: The compound benzotriazole and alkyl halides were dissolved in DMF solution and kept at 30–40 s in a microwave oven at 60% power. After completion of the reaction, the dark red mass was poured into ice-cold water and worked up as described earlier. Compound **2a: 4′benzotriazol-1-yl-methyl-biphenyl-2-carbonitrile** was obtained by using benzotriazole (1 g, 8.39 mmol), 4-(2-cyano-phenyl)-benzyl bromide (2.28 g), and K_2CO_3 (3.47 g). ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 6.1 (s, 2H, Bz-N-CH_2-), 7.41–7.5 (m, 3H, Ar-H), 7.52–7.62 (m, 4H, Ar-H), 7.75–7.8 (t, 1H, Bz-H), 7.92–7.99 (d, 1H, J = 6 Hz, Bz-H), 8.07–8.11 (d, 1H, J = 5 Hz, Bz-H). Anal. Calcd for $C_{20}H_{14}N_4$: C, 77.40; H, 4.55; N, 18.05; Found: C, 77.21; H, 4.38; N, 18.13; compound **2b**: **1-(4-nitro-benzyl)-1H-benzotriazole** was obtained by using benzotriazole (1 g, 8.39 mmol), 4-nitro benzyl bromide (1.81 g), and K_2CO_3 (3.47 g). Purification: silica gel column chromatography using *n*-hexane/ethyl acetate: 7:3. ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 5.82–5.9 (s, 2H, –CH_2–), 6.82 (d, 2H, J = 3 Hz, Ar-H), 7.36–7.46 (m, 2H, Ar-H), 7.76–7.81 (d, 1H, J = 8 Hz, Bz-H), 7.9–7.94 (t, 1H, Bz-H), 8.04–8.06 (d, 1H, J = 8 Hz, Bz-H). Anal. Calcd for $C_{13}H_8N_4O_3$: C, 58.21; H, 3.01; N, 20.89; Found: C, 58.12; H, 2.98; N, 20.91; compound **2c: 1-(2-bromo-4,5-dimethoxy-benzyl)1H-benzotriazole** was obtained by using benzotriazole (1 g, 8.39 mmol), 1-bromo-2-(bromomethyl)-4,5-dimethoxybenzene (2.46 g), and K_2CO_3 (3.47 g).^{1}H NMR ($CDCl_3$, 400 MHz, δ): 5.82-5.9 (s, 2H, -CH_2-), 6.82 (d, 2H, J = 3 Hz, Ar-H), 7.36–7.46(m, 2H, Ar-H), 7.76–7.81 (d, 1H, J = 8 Hz, Bz-H), 7.9–7.94 (t, 1H, Bz-H), 8.04–8.06 (d, 1H, J = 8 Hz, Bz-H). Anal. Calcd for $C_{15}H_{14}N_3BrO_2$: C, 51.74; H, 4.05; N, 12.07; Found: C, 51.46; H, 4.18; N, 12.18; compound **2d: 2-benzotriazole-1yl-1-(4-chloro-phenyl)-ethanone** was obtained by using benzotriazole (1 g, 8.39 mmol), 2-bromo-1-(4-chlorophenyl)ethanone (1.95 g), and K_2CO_3 (3.47 g). ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 6.6 (s, 2H, –CH_2-CO–), 6.82 (d, 2H, J = 3 Hz, Ar-H), 7.41–7.48 (m, 1H, Bz-H), 7.52–7.57 (t, 1H, Bz-H), 7.71–7.77 (d, 2H, J = 9 Hz, Ar-H), 7.81–7.83 (d, 1H, J = 8 Hz, Bz-H), 8.1–8.21 (dd, 3H, J = 8 Hz, J = 8 Hz, Ar-H). Anal. Calcd for C_{14} $H_{10}N_3OCl$: C, 61.89; H, 3.71; N, 15.47; Found: C, 61.76; H, 3.82; N, 15.38. Compound **2e: 1-((5-methylbenzo[1,3]dioxol-6-yl)methyl)-1*H*-benzotriazole** was obtained by using benzotriazole (1 g, 8.39 mmol), 2-bromo-1-(4-chlorophenyl)ethanone (1.54 g), and K_2CO_3 (3.47 g). ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 2.23 (s, 3H, Ar-CH_3), 6.2 (s, 2H, –CH_2-CO–), 5.8 (s, 2H, –O-CH_2-O–), 6.72 (d, 2H, J = 12 Hz, Ar-H), 6.82–6.9 (m, 1H, Ar-H), 7.24–7.46 (d, 1H, Bz-H), 7.6–7.68 (t, 1H, Bz-H), 7.81–7.83 (d, 1H, Bz-H), Anal. Calcd for $C_{15}H_{13}N_3O_2$: C, 67.40; H, 4.90; N, 15.72; Found: C, 67.38; H, 4.89; N, 15.65; compound **2f: 1-propyl-1H-benzotriazole** was obtained by using benzotriazole (1 g, 8.39 mmol), *n*-propyl bromide (1.03 g), and K_2CO_3 (3.47 g). ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 0.94 (t, 3H, -CH_3), 2.1 (m, 2H, CH_3-CH_2), 4.8 (t, 2H, N-CH_2-), 7.4–7.56 (dt, 2H, J = 10 Hz, Bz-H), 7.81–7.92 (d, 2H, J = 8 Hz, Bz-H). Anal. Calcd for $C_9H_{11}N_3$: C, 67.06; H, 6.88; N, 26.07; Found: C, 66.97; H, 6.45; N, 26.12; **2g: 1-butyl-1H-benzotriazole** was obtained by using benzotriazole (1 g, 8.39 mmol), *n*-butyl bromide (1.14 g), and K_2CO_3 (3.47 g). ^{1}H NMR ($CDCl_3$, 400 MHz, δ): 0.92 (t, 3H, -CH_3), 1.84 (m, 2H, CH_3-CH_2), 2.1–2.4 (m, 2H, -CH_2-), 4.8–4.86 (t, 2H, -N-CH_2-), 7.34–7.46 (dt, 2H, J = 6 Hz, Bz-H), 7.86–7.8–7.98 (d, 2H, Bz-H). Anal. Calcd for $C_{14}H_{13}N_3$: C, 68.54; H, 7.48; N, 23.98; Found: C, 68.42; H, 7.34; N, 23.67.
18. X-ray crystal structure analysis: A single crystal of suitable size is chosen for X-ray study. All the measurements were made on a Rigaku AFC7S diffractometer with graphite monochromated radiation (*MoK*α). The data were collected using the $\omega - 2\theta$ scan technique and were reduced by teXsan[13] data reduction program. Lorentz and polarization corrections were applied. The structure was solved by using direct methods (SHELXS-97)[14] and refined by least-squares method (SHELXL-97).[15] The full crystallographic details have been deposited at Cambridge Crystallography Data Center (CCDC No. 277414).
19. *Biology*: Bacteria and fungal species used were obtained from Microbiology Department, University of Mysore, India. Namely, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Xanthomonas campestris pvs*, *Xanthomonas oryzae*, *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, *Trichoderma* species,and *Fusarium monaliforme*. The bacterial strains were maintained on LB agar medium and the filamentous fungi were maintained on potato dextrose agar (PDA) medium at 28 °C. The disk diffusion method[16] was used to determine the antimicrobial activity of synthesized compounds. Paper disks with only DMSO were used as negative controls. The bacteria were grown in LB broth, centrifuged at 10,000 rpm for 5 min, the pellet was dissolved in double distilled water and used to inoculate the plates. For the filamentous fungi, the inoculum was prepared with the spores derived from 5 to 15 days culture on PDA medium. The mycelia were covered with 10 mL distilled water and the conidia were scraped using sterile pipette. The spores were recovered after filtration on sterile absorbent cotton and resuspended in sterile distilled water. The cell density of each inoculum was adjusted with hemocytometer in order to obtain a final concentration of approximately 10^4 and 10^6 CFU/mL for the bacteria and filamentous fungi, respectively. Nystatin (Himedia) was used as positive control for fungi, and streptomycin and tetracycline for

bacteria. Each disk contained 10 μg standard drugs and 25 μg synthesized compounds. Plates were first kept at 4 °C for at least 2 h to allow the diffusion of chemicals and then incubated at 28 °C. Inhibition zones were measured after 24 h of incubation for bacteria and after 48 h of incubation for fungi.