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SYNTHESIS AND ANTI-MICROBIAL EVALUATION OF SOME NOVEL 1,2,4 - TRIAZOLE DERIVATIVES

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Abstract: Triazoles with different substituent groups are found to possess diverse applications in the field of medicine and industry. A series of 4-(substituted ethanoyl)amino-3-mercapto-5-(4-nitro)phenyl-1,2,4-triazoles (NU-1 to NU-15) were synthesized as novel antimicrobial agents starting from 4-nitrobenzoic acid. The chemical structures of these newly synthesized compounds were elucidated by IR, ^{1}H NMR, ^{13}C NMR, FAB^{+}-MS spectral data and elemental analysis. Their antimicrobial activities against *Staphylococcus aureus* (ATCC-25923), *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-8739), *Bacillus substilis* (ATCC-6633), *Candida albicans* (MTCC-227), *Aspergillus niger* (MTCC-3323) and *Fusarium oxysporum* (MTCC-2087) were investigated.

Keywords: 1,2,4-triazoles, cyclization, zone of inhibition, antibacterial, antifungal.

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medicinal use, at the same time the emergence of resistance developed against old and new antibiotics in the last decades revealed a substantial need for new classes of antimicrobial agents. There is really perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms of action, which are distinct from those of well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant.

Through the various molecules designed and synthesized for this aim, in recent years, active research has been initiated on heterocycles and the chemistry of 1,2,4-triazoles has received considerable attention owing to their synthetic and effective biological importance. 1,2,4-Triazole moiety has been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, antimicrobial and antimycotic agents such as fluconazole, itraconazole, voriconazole (1). There are many marketed drugs containing the 1,2,4-triazole group e.g., triazolam, alprazolam, etizolam and furacyclin. From the literature, it may be predicted that 1,2,4-triazole moiety represents important pharmacophore and play a vital role in medicinal agents. A degree of respectability has been bestowed upon 1,2,4-triazole derivatives due to their wide range of biological activities such as antibacterial (2), antifungal (3), antitubercular (4), anticancer (5) anti-tumor (6), anti-inflammatory (7), anticonvulsant (8), anxiolytic (9), antidepressant (10) and hypoglycemic properties (11). Certain 1,2,4-triazole derivatives are also found to be Openers of Large-Conductance Ca^{2+}-Activated Potassium (Maxi-K) Channels (12).

In the design of new drugs, the combination of different pharmacophores frame may lead to compounds with interesting biological profiles.

As secondary amine incorporated heterocycles like thiazole, oxadiazole, and 1,2,4-triazole displayed varied pharmacological properties. Prompted

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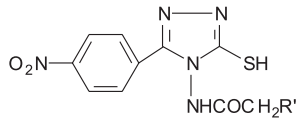
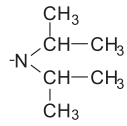
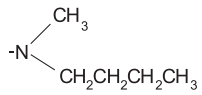
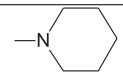
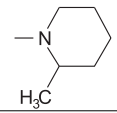
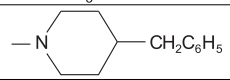
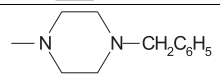
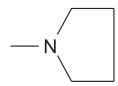
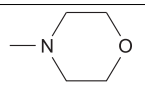
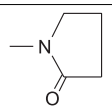
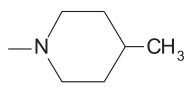
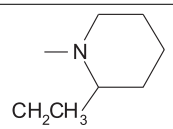
Table 1. List of the synthesized compounds.



Compound code	R'	Yield (%)
NU-1	$-N(CH_2CH_3)_2$	56
NU-2	$-N(CH_2CH_2CH_3)_2$	62
NU-3		66
NU-4	$-N(CH_2CH_2CH_2CH_3)_2$	38
NU-5		65
NU-6		53
NU-7		62
NU-8		49
NU-9		35
NU-10		32
NU-11		52
NU-12		34
NU-13		51
NU-14		56
NU-15	$-N(CH_2CH_2CH_2CH_2CH_3)_2$	47

by these investigations we synthesized compounds containing 1,2,4-triazole with attached secondary amine group and evaluated them for their antimicrobial activity.

EXPERIMENTAL

Chemistry

Melting points were recorded in open capillary tubes and are uncorrected. IR spectra were recorded in KBr discs in Nicolet-6700 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400 MHz apparatus using DMSO-d_6 as the solvent. Mass spectra were recorded on Jeol Sx 102/DA-600 mass spectrometer/Data system using fast atom bombardment (FAB) technique and nitrogen analysis was done on elemental analyzer Elementar Vario EL III Carlo Erba 1108. The purity of the synthesized compounds was confirmed by thin layer chromatography (TLC) using silica gel G. Visualization was done using iodine vapors or sulfuric acid (30% v/v).

A series of 1,2,4-triazole derivatives were synthesized using different secondary amines and 4-nitrobenzoic acid. Synthesis of the 1,2,4-triazole derivatives is shown in Scheme 1.

Synthesis of methyl ester of 4-nitrobenzoic acid (1)

To 4-nitrobenzoic acid (0.1 mol) in methanol (100 mL) in a round bottom flask conc. sulfuric acid (5.7 mL) was added. The mixture was refluxed for 4–6 h. An excess of methanol was distilled off and after cooling, the content was transferred to separating funnel containing 100 mL of distilled water. The synthesized ester was extracted several times with carbon tetrachloride (30 mL). The combined organic layers were washed with 20% solution of sodium bicarbonate to remove any unreacted acid. After washing with distilled water, the organic layer was dried over anhydrous $MgSO_4$. Carbon tetrachloride was then distilled off under reduced pressure giving ester (**1**), which was recrystallized from absolute ethanol. Yield 96 %, m.p. 96–98°C.

Synthesis of acid hydrazide of 4-nitrobenzoic acid methyl ester (2)

To hydrazine hydrate (99%) (5.7 mL, 0.15 mol) in a flat bottom flask a solution of **1** (0.1 mol) in ethanol was added dropwise with gentle stirring. After complete addition, the mixture was transferred into a round bottomed flask and refluxed for 4–6 h. Ethanol was distilled off under reduced pressure. The precipitate of acid hydrazide (**2**) was filtered and recrystallized from ethanol. Yield 94%, m.p. 216–218°C.

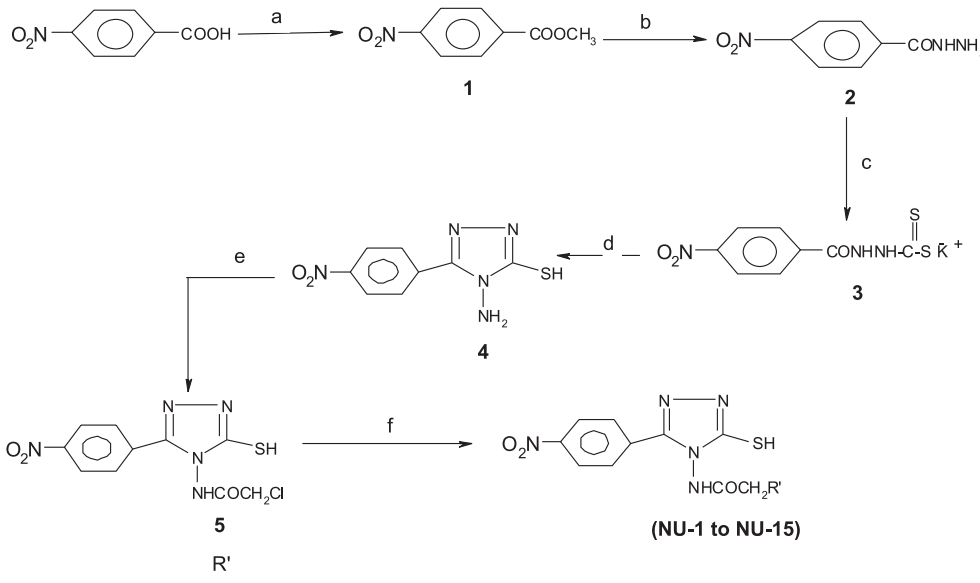
Synthesis of potassium 3-(4-nitrobenzoyl) dithiocarbazate (3)

A mixture of potassium hydroxide (0.15 mol), 100 mL of absolute ethanol and (0.1 mol) of **2** was

Table 2. Physical properties of the synthesized compounds.

Compound code	Molecular formula	Molecular weight	Melting point (°C)	% Nitrogen		R_f[a]
				Calcd.	Found	
NU-1	$C_{14}H_{18}N_6SO_3$	350	185-187	23.98	23.67	0.58
NU-2	$C_{16}H_{22}N_6SO_3$	378	226-228	22.21	22.23	0.60
NU-3	$C_{16}H_{22}N_6SO_3$	378	232-234	22.21	21.87	0.61
NU-4	$C_{18}H_{26}N_6SO_3$	406	220-222	20.67	20.74	0.73
NU-5	$C_{15}H_{20}N_6SO_3$	364	204-206	23.06	23.11	0.63
NU-6	$C_{15}H_{18}N_6SO_3$	362	184-186	23.19	23.02	0.61
NU-7	$C_{16}H_{20}N_6SO_3$	376	144-146	22.23	23.82	0.65
NU-8	$C_{22}H_{24}N_6SO_3$	452	216-218	18.57	18.72	0.78
NU-9	$C_{21}H_{23}N_7SO_3$	453	182-184	21.62	21.91	0.81
NU-10	$C_{14}H_{16}N_6SO_3$	348	198-200	24.12	24.62	0.64
NU-11	$C_{14}H_{16}N_6SO_4$	364	162-164	23.06	22.23	0.67
NU-12	$C_{14}H_{14}N_6SO_4$	362	206-208	23.19	24.01	0.66
NU-13	$C_{16}H_{20}N_6SO_3$	376	152-154	21.92	21.10	0.69
NU-14	$C_{17}H_{22}N_6SO_3$	390	144-146	20.15	20.67	0.75
NU-15	$C_{20}H_{30}N_6SO_3$	434	242-244	20.15	20.01	0.87

[a]Solvent system used: ethyl acetate : petroleum ether (1:1, v/v)



Scheme 1. Synthesis of the title compounds (**NU-1** to **NU-15**)
Reagents and conditions: a) CH_3OH and conc. H_2SO_4, b) NH_2 NH_2H_2O, c) CS_2 and alc. KOH, d) NH_2 NH_2H_2O and C_2H_5OH, e) $ClCH_2COCl$, f) different secondary amines

treated with (0.15 mol) of carbon disulfide. This mixture was diluted with 75 mL of absolute ethanol and stirred for 12–16 h. The solvent was distilled off under reduced pressure. The salt, prepared as described above, was obtained in nearly quantitative yield and was employed without further purification.

Synthesis of 4-amino-3-mercapto-5-(4-nitro)phenyl-1,2,4-triazole (4)

A suspension of (0.1 mol) of **3** in absolute alcohol, (0.2 mol) of 99% hydrazine hydrate and 6 mL of water was refluxed for 2 to 3 h. The color of the reaction mixture changed to green with the evolu-

Table 3. Spectral data of synthesized compounds.

Compd.	IR (KBr) (cm^{-1})	^{1}H NMR (DMSO-d_6) (δ, ppm)	^{13}C NMR (DMSO-d_6) (δ, ppm)
NU-1	1407 (C-N), 1520 (C=N), 3103.8 (Ar-CH), 2550 (S-H), 1668 (C=O), 1530 (N=O asymmetric str), 1327 (N=O symmetric str), 870 (Ar-C-N)	1.09 (t, 2x3H, CH_3), 2.63 (q, 2x2H, CH_2), 3.08 (s, 2H, $COCH_2$), 7.44 (d, *J* = 9.1, 2H, Ar-H), 7.92 (d, *J* = 9.1, 2H, Ar-H), 9.92 (s, 1H, SH), 10.25 (s, 1H, CONH)	165.5, 147, 146, 141.8, 127.4, 125.79, 48.5, 41.2, 21.3, 10.9
NU-2	1350 (C-N), 1531 (C=N), 3100 (Ar-CH), 2550 (S-H), 1668 (C=O), 1530 (N=O asymmetric str),1330 (N=O symmetric str), 870 (Ar-C-N), 3350(N-H str), 1603 (Ar-C=C)	1.12 (t, 2x3H,CH_3), 2.21 (m, 8H, 2xCH_2), 3.09 (s, 2H, $COCH_2$), 7.56 (d, *J* = 8.9, 2H, Ar-H), 7.97 (d, J = 9.0, 2H, Ar-H), 9.78 (s, 1H, SH), 10.06 (s, 1H, CONH)	165, 147, 142, 141, 127.4, 25.9, 59.9, 156.3, 22.5, 11.7
NU-3	1407 (C-N), 1531 (C=N), 3067 (Ar-CH), 2510 (S-H), 1686 (C=O), 1531 (N=O asymmetric str),1332 (N=O symmetric str), 870 (Ar-C-N), 3400(N-H str), 1604 (Ar-C=C)	1.19 (d, *J* = 8.8, 12H, 4xCH_3), 1.82 (m, 2H, CH), 2.89 (s, 2H, $COCH_2$), .68 (d, *J*=7.8, 2H, Ar-H), 7.92 (d, *J* = 7.9, 2H, Ar-H), 79.87 (s, 1H, SH), 10.15 (s, 1H, CONH)	160.2, 147, 142, 141, 127.2, 123.1, 51, 46, 29
NU-4	1410 (C-N), 1531 (C=N), 3020 (Ar-CH), 2520 (S-H), 1686 (C=O), 1520 (N=O asymmetric str), 1332 (N=O symmetric str), 870 (Ar-C-N), 3401(N-H str), 1604 (Ar-C=C)	1.09 (t, 2x3H, CH_3), 1.59 (m, 8H, 2xCH_2), 2.54 (m, 4H, 2xCH_2), 3.23 (s, 2H, $COCH_2$), 7.80 (d, *J* = 8.7, 2H, Ar-H), 7.90 (d, *J* = 8.4 2H, Ar-H), 9.92 (s, 1H, SH), 10.09 (s, 1H, CONH)	166, 147, 142.4, 141, 127.9, 125, 54, 46.9, 37.3, 19.7, 13.9
NU-5	1410 (C-N), 1531 (C=N), 3030 (Ar-CH), 2520 (S-H), 1671 (C=O), 1520 (N=O asymmetric str), 1334 (N=O symmetric str), 870 (Ar-C-N), 3400(N-H str), 1604 (Ar-C=C)	1.10 (t, 3H, CH_3), 1.48 (m, 4H, 2xCH_2), 2.59 (m, 5H, CH_3 & CH_2), 3.11 (s, 2H, $COCH_2$), 7.84 (d, *J* = 8.6, 2H, Ar-H), 7.95 (d, *J* = 8.4, 2H, Ar-H), 9.94 (s, 1H, SH), 10.17 (s, 1H, CONH)	66.1, 147, 142.4, 141, 128.3, 127.9, 79, 58, 1 39.2, 32.8, 19.6, 13.9
NU-6	1401 (C-N), 1540 (C=N),, 3123 (Ar-CH), 2520 (S-H), 1671 (C=O), 1520 (N=O asymmetric str), 1334 (N=O symmetric str), 842 (Ar-C-N), 3311.6 (N-H str), 1603 (Ar-C=C)	1.50 (m, 10H, piper.), 3.21 (s, 2H, $COCH_2$), 7.68 (d, *J* = 8.7, 2H, Ar-H), 8.11 (d, *J* = 8.5, 2H, Ar-H), 9.83 (s, 1H, SH), 10.13 (s, 1H, CONH)	68.9, 147, 142, 141, 1127.6, 125.1, 57, 53.6, 22.6, 20.8
NU-7	1411.3 (C-N), 1528.7 (C=N),, 3050 (Ar-CH), 2540 (S-H), 1678.1 (C=O), 1528.7 (N=O asymmetric str), 1323.5 (N=O symmetric str), 842 (Ar-C-N), 3311.6 (N-H str), 1603 (Ar-C=C)	1.18 (s, 3H, CH_3- piper.), 1.96 (m, 11H, CH, CH_2,), 7.62 (d, *J* = 8.3, 2H, Ar-H), 8.24 (d, *J* = 8.9, 2H, Ar-H), 9.77 (s, 1H, SH), 10.11 (s, 1H, CONH)	168.9, 147, 142, 141, 27.6, 125.1, 54, 51, 143.3, 32.2, 30, 23, 19.8
NU-8	1411 (C-N), 1532.6 (C=N),, 3020 (Ar-CH), 2520 (S-H), 1666 (C=O), 1528.7 (N=O asymmetric str), 1328.9 (N=O symmetric str), 842.4 (Ar-C-N), 3311.6 (N-H str), 1603 (Ar-C=C), 1470.7 (CH_2 bending of piperidine)	1.50 (m, 11H, CH_2), 3.30 (s, 2H, $COCH_2$), 7.81 (m, 7H, Ar-H), 7.96 (d, *J* = 7.9, 2H, Ar-H), 9.91 (s, 1H, SH), 10.87 (s, 1H, CONH)	166, 148, 142.3, 141, 139.8, 128.3, 127.9, 126.4, 58, 49, 39.9, 31.7, 28.5
NU-9	1410.7 (C-N), 1526.9 (C=N),, 3000 (Ar-CH), 2520 (S-H), 1681.7 (C=O), 1532.1 (N=O asymmetric str), 1326.9 (N=O symmetric str), 842.3 (Ar-C-N),1604 (Ar-C=C), 1012 (CH_2 bending of piperazine)	2.77 (m, 8H, CH_2-piperazin), 3.44 (s, 2H, $COCH_2$), 3.79 (s, 2H, Ar-CH_2), 7.59 (m, 7H, Ar-H), 7.95 (d, *J* = 8.3, 2H, Ar-H), 9.61 (s, 1H, SH), 10.35 (s, 1H, CONH)	166, 148, 142, 141, 135, 128.9, 128.2, 127.4, 127, 126, 61.4, 58, 55.6, 55
NU-10	1410.6 (C-N), 1531.4 (C=N),, 3100 (Ar-CH), 2520 (S-H), 1664.6 (C=O), 1510 (N=O asymmetric str), 1325.4 (N=O symmetric str), 842.3 (Ar-C-N),1604 (Ar-C=C), 3305 (N-H), 1468.5 (CH_2 bending of pyrrolidine)	1.38 (t, 2x2H, CH_2-pyrr.), 1.57 (t, 2x2H, CH_2-pyrr.), 3.91 (s, 2H, $COCH_2$), 7.60 (d, *J* = 9.9, 2H, Ar-H), 8.32 (d, *J* = 8.9, 2H, Ar-H), 9.88 (s, 1H, SH), 10.16 (s, 1H, CONH)	166, 148, 142, 141, 127.4, 126, 58, 51, 23.1

Table 3. Cont.

Compd.	IR (KBr) (cm^{-1})	^{1}H NMR (DMSO-d_6) (δ, ppm)	^{13}C NMR (DMSO-d_6) (δ, ppm)
NU-11	1410.4(C-N), 1533.8 (C=N),, 3100 (Ar-CH), 2540 (S-H), 1699.5 (C=O), 1510 (N=O asymmetric str), 1325.4 (N=O symmetric str), 842.3 (Ar-C-N), 1604.6 (Ar-C=C), 1067.8 (Cyclic ether) 1501.4 (CH_2 bending of morpholine),	2.57 (t, 2x2H, CH_2-morph.), 3.50 (m, 4H, CH_2-morph.), 3.63 (s, 2H, $COCH_2$), 7.67 (d, *J* = 10.0, 2H, Ar-H), 8.26 (d, *J* = 9.8, 2H, Ar-H), 9.83 (s, 1H, SH), 10.17 (s, 1H, CONH)	166, 148, 142, 141, 127, 119.7, 63.3, 59.1, 55
NU-12	1412.8(C-N), 1534.4 (C=N),, 3100 (Ar-CH), 2520 (S-H), 1664.1 (C=O), 1510 (N=O asymmetric str), 1330 (N=O symmetric str), 842.3 (Ar-C-N),1603.3 (Ar-C=C), 1700.8 (á-butyrolactam) 1469.3 (CH_2 bending of pyrrolidinone),	2.33 (m, 4H, CH_2-pyrr.), 3.73 (m, 4H, CH_2-pyrr. & $COCH_2$), 7.79 (d, *J* = 9.1, 2H, Ar-H), 8.44 (d, *J* = 9.5, 2H, Ar-H), 9.78 (s, 1H, SH), 10.18 (s, 1H, CONH)	177.8, 148, 142, 141, 127.9, 119.8, 72, 52, 40.2, 39.2, 20.8
NU-13	1323.8(C-N), 1534.5 (C=N),, 3020 (Ar-CH), 2540 (S-H), 1660 (C=O), 1525 (N=O asymmetric str), 1323.8 (N=O symmetric str), 842.3 (Ar-C-N),1604.5 (Ar-C=C), 3350 (N-H) 1472.6 (CH_2 bending of piperdine),	1.19 (s, 3H, CH_3- piper.), 1.96 (m, 14H, CH, CH_2, & CH_3), 7.04 (m, 7H, Ar-H), 8.24 (d, *J* = 8.8, 2H, Ar-H), 9.89 (s, 1H, SH), 10.18 (s, 1H, CONH)	170, 147, 143, 142, 127.8, 126.2, 53, 46.5, 30.6, 28.6, 21.8
NU-14	1322 (C-N), 1530 (C=N),, 3100 (Ar-CH), 2520 (S-H), 1697.5 (C=O), 1524 (N=O asymmetric str),1322 (N=O symmetric str), 840 (Ar-C-N),1603.8 (Ar-C=C), 3371.8 (N-H) 1469.5 (CH_2 bending of piperdine),	1.78 (m, 13H, CH_2 & CH_3), 2.92 (m, 3H, CH, CH_2), 7.72 (d, *J* = 9.7, 2H, Ar-H), 8.61 (d, *J* = 8.5, 2H, Ar-H), 9.97 (s, 1H, SH), 10.08 (s, 1H, CONH)	167.1, 148, 147, 141.8, 127.9, 119.7, 57.5, 56, 50, 27.8 26.4, 26.2, 22.4, 9.9
NU-15	1326.4 (C-N), 1534.9 (C=N),, 3000 (Ar-CH), 2540 (S-H), 1660 (C=O), 1520 (N=O asymmetric str),1330 (N=O symmetric str), 870 (Ar-C-N),1601.6 (Ar-C=C), 3350 (N-H)	1.70 (m, 18H, CH_2 & CH_3), 2.67 (m, 4H, CH_2), 3.81 (s, 2H, $COCH_2$), 7.84 (d, *J* = 8.3, 2H, Ar-H), 8.38 (d, *J* = 8.0, 2H, Ar-H), 9.96 (s, 1H, SH), 10.12 (s, 1H, CONH)	165.1, 150, 147, 135.4, 127.9, 123.5, 56, 52.8, 30.1, 23, 21.7, 14

tion of hydrogen sulfide gas and a homogenous solution resulted. Cold distilled water (100 mL) was added and the solution was acidified with conc. HCl. The precipitated solid was filtered, washed with 2 × 30 mL portions of cold water, and recrystallized. Yield 62%, m.p. 180–182°C.

Synthesis of 4-chloroacetylamino-3-mercapto-5-(4-nitro)phenyl 1,2,4-triazole (5)

Compound **4** (0.1 mol) was taken in a 50 mL of dioxane in a two necked round bottom flask fitted with reflux condenser and a separating funnel. Chloroacetyl chloride (8.75 mL, 0.11 mol) was taken in dioxane (25 mL), in the separating funnel. The chloroacetyl chloride was added in small portions to the vessel. After complete addition, the content of the flasks was refluxed for 1 h. After cooling, the content was poured on crushed ice. The precipitated product was filtered and washed several times with ice cold distilled water. Yield 58%, m.p. 132–134°C.

Synthesis of title compounds (NU-1 to NU-15)

Compound **5** (0.025 mol) and respective amine (0.030 mol) along with the triethylamine (0.030 mol) in a round bottomed flask in benzene (75 mL) were refluxed for 3–4 h. The precipitated triethylamine hydrochloride was separated out. The organic layer was washed several times with distilled water to remove last traces of hydrochloride. Benzene was distilled off under vacuum and the crude product was separated and purified by repeated crystallization from appropriate solvents (Tables 1–3).

ANTIMICROBIAL EVALUATION

The antibacterial activity of the synthesized compounds were tested against *Staphylococcus aureus* (ATCC-25923), *Bacillus subtilis* (ATCC-6633) (Gram positive) and *Pseudomonas aeruginosa* (ATCC-27853), *Escherichia coli* (ATCC-8793) (Gram negative) bacterial strains (Table 4). Antifungal activity of the synthesized compounds were tested against *Aspergillus niger* (MTCC-3323), *Candida albicans* (MTCC-227) and *Fusarium oxysporum* (MTCC-2087) fungal strains (Table 5).

Antibacterial activity

The newly synthesized compounds were screened for their antibacterial activity against

Table 4. Antibacterial activity of the synthesized compounds (in mm).

Compd.	*S. aureus*			*B. subtilis*			*P. aeruginosa*			*E. coli*		
				µg/mL						µg/mL		
	70	50	30	70	50	30	70	50	30	70	50	30
NU-1	8.36 ± 0.55	7.33 ± 0.58	6.90 ± 0.70	7.26 ± 0.74	6.20 ± 0.46	6.06 ± 0.80	8.16 ± 0.56	7.13 ± 0.61	6.20 ± 0.46	8.93 ± 0.80	7.13 ± 0.61	6.83 ± 1.00
NU-2	10.10 ± 0.80	9.16 ± 0.55	8.20 ± 0.60	9.93 ± 0.61	8.96 ± 0.70	7.03 ± 0.64	10.16 ± 0.55	9.16 ± 0.70	8.16 ± 0.57	9.13 ± 0.70	8.13 ± 0.56	7.06 ± 0.71
NU-3	8.50 ± 0.40	7.20 ± 0.46	6.23 ± 0.42	8.20 ± 0.62	10.16 ± 0.47	8.30 ± 0.56	8.40 ± 0.30	7.23 ± 0.56	6.16 ± 0.56	8.20 ± 0.46	7.40 ± 0.30	7.30 ± 0.46
NU-4	11.23 ± 0.55	10.43 ± 0.42	9.23 ± 0.55	11.33 ± 1.26	7.23 ± 0.56	6.16 ± 0.56	12.13 ± 0.95	10.16 ± 0.47	9.36 ± 0.75	10.23 ± 0.55	9.26 ± 0.55	8.36 ± 0.75
NU-5	8.20 ± 0.70	7.30 ± 0.56	6.56 ± 0.40	8.16 ± 0.56	7.23 ± 0.58	6.37 ± 0.60	9.30 ± 0.56	8.20 ± 0.53	7.00 ± 0.56	7.26 ± 0.56	6.61 ± 0.46	6.33 ± 0.21
NU-6	9.13 ± 0.56	8.23 ± 0.56	7.26 ± 0.50	7.36 ± 0.35	6.30 ± 0.56	6.13 ± 0.61	7.13 ± 0.56	6.30 ± 0.36	6.13 ± 0.61	7.23 ± 0.51	6.43 ± 0.60	6.23 ± 0.65
NU-7	7.13 ± 0.61	6.23 ± 0.51	6.20 ± 0.53	8.13 ± 0.56	7.16 ± 0.47	6.23 ± 0.65	8.26 ± 1.36	7.40 ± 0.30	6.23 ± 0.58	8.13 ± 0.56	7.10 ± 0.56	6.93 ± 0.85
NU-8	7.16 ± 0.70	6.93 ± 0.61	6.13 ± 0.61	9.30 ± 0.56	8.10 ± 0.46	7.43 ± 0.60	9.13 ± 0.56	8.86 ± 0.56	7.23 ± 0.58	8.23 ± 0.61	7.06 ± 0.70	6.40 ± 0.80
NU-9	8.23 ± 0.56	7.16 ± 0.75	6.30 ± 0.56	7.13 ± 0.56	6.10 ± 0.56	6.06 ± 0.05	8.00 ± 0.75	7.10 ± 0.56	6.26 ± 0.50	9.40 ± 0.56	8.60 ± 0.56	7.06 ± 0.56
NU-10	6.90 ± 0.70	6.23 ± 0.42	6.10 ± 0.10	6.30 ± 0.56	6.20 ± 0.46	6.10 ± 0.10	6.33 ± 0.21	6.17 ± 0.56	6.10 ± 0.10	6.76 ± 0.61	6.33 ± 0.21	6.16 ± 0.70
NU-11	10.10 ± 0.75	9.20 ± 0.46	8.30 ± 0.44	10.30 ± 0.56	8.23 ± 0.70	7.26 ± 0.68	11.43 ± 0.42	10.20 ± 0.56	9.86 ± 0.56	9.13 ± 0.95	8.86 ± 0.56	7.10 ± 0.76
NU-12	7.10 ± 0.46	6.76 ± 0.61	6.13 ± 0.65	6.76 ± 0.61	6.40 ± 0.46	6.13 ± 0.61	6.40 ± 0.46	6.17 ± 0.56	6.13 ± 0.61	7.06 ± 0.65	6.33 ± 0.21	6.06 ± 0.71
NU-13	8.26 ± 0.65	7.06 ± 0.75	6.30 ± 0.65	7.16 ± 0.56	6.16 ± 0.56	3.13 ± 0.56	7.16 ± 0.65	6.96 ± 0.65	6.20 ± 0.56	7.03 ± 0.56	6.30 ± 0.56	6.13 ± 0.45
NU-14	9.13 ± 0.56	8.16 ± 0.75	7.03 ± 0.56	7.06 ± 0.56	6.36 ± 0.64	6.10 ± 0.75	8.56 ± 0.56	7.16 ± 0.75	6.63 ± 0.56	9.16 ± 0.56	8.20 ± 0.82	7.30 ± 0.46
NU-15	8.16 ± 0.47	7.23 ± 0.56	6.10 ± 0.10	6.90 ± 0.70	6.33 ± 0.21	6.10 ± 0.10	7.20 ± 0.62	6.46 ± 0.45	6.13 ± 0.41	7.30 ± 0.60	6.36 ± 0.64	6.16 ± 0.47
Van.			18.20 ± 0.30			17.43 ± 0.35						
Amik.									22.10 ± 0.36			21.27 ± 0.56

Van. = vancomycin, Amik. = amikacin

Table 5. Antifungal activity of the synthesized compounds (in mm).

Compd.	*C. albicans*			*A. niger*			*F. oxysporum*		
	µg/mL								
	70	50	30	70	50	30	70	50	30
NU-1	8.10 ± 0.56	7.03 ± 0.90	6.96 ± 1.23	9.17 ± 0.70	8.33 ± 1.30	7.10 ± 0.56	7.20 ± 0.62	6.23 ± 0.65	6.13 ± 0.65
NU-2	10.33 ± 0.56	9.23 ± 0.63	8.26 ± 0.65	11.13 ± 0.65	10.23 ± 0.56	9.03 ± 0.75	9.30 ± 0.75	8.16 ± 0.65	7.10 ± 0.56
NU-3	9.16 ± 0.75	8.13 ± 0.56	7.46 ± 0.45	10.03 ± 0.55	9.13 ± 0.65	8.13 ± 0.56	8.31 ± 0.56	7.16 ± 0.50	6.23 ± 0.65
NU-4	11.06 ± 0.65	10.16 ± 0.56	9.23 ± 0.70	14.06 ± 0.65	12.16 ± 0.65	11.13 ± 0.51	10.10 ± 0.72	8.10 ± 1.10	7.16 ± 0.65
NU-5	8.23 ± 0.55	7.10 ± 0.56	6.16 ± 0.81	10.13 ± 0.70	9.16 ± 0.70	8.20 ± 0.56	7.20 ± 0.66	6.23 ± 0.55	6.10 ± 0.10
NU-6	7.33 ± 0.65	6.23 ± 0.65	6.13 ± 0.65	7.16 ± 0.65	6.33 ± 0.21	6.13 ± 0.65	8.16 ± 0.75	7.20 ± 0.66	6.13 ± 1.20
NU-7	9.17 ± 0.65	8.60 ± 0.46	7.40 ± 0.46	8.10 ± 0.85	7.50 ± 0.82	6.13 ± 0.65	9.60 ± 0.46	8.10 ± 0.56	7.03 ± 0.55
NU-8	8.16 ± 0.65	7.33 ± 0.21	6.16 ± 0.70	7.03 ± 0.65	6.26 ± 0.65	6.16 ± 0.70	6.20 ± 0.46	6.17 ± 0.56	6.10 ± 0.10
NU-10	7.13 ± 0.70	6.20 ± 1.02	6.13 ± 0.65	9.13 ± 0.70	8.13 ± 0.75	7.16 ± 0.61	6.33 ± 0.21	6.23 ± 0.21	6.10 ± 0.10
NU-11	6.20 ± 0.46	6.17 ± 0.56	6.10 ± 0.10	7.03 ± 0.56	6.20 ± 0.66	6.13 ± 0.65	6.76 ± 0.61	6.33 ± 0.55	6.23 ± 0.65
NU-12	10.16 ± 0.65	9.06 ± 0.65	8.21 ± 0.66	12.06 ± 0.65	10.17 ± 0.65	9.20 ± 0.75	8.03 ± 0.55	7.06 ± 0.65	6.80 ± 0.56
NU-13	8.16 ± 0.65	7.13 ± 0.55	6.14 ± 0.56	7.16 ± 0.75	6.76 ± 0.61	6.23 ± 0.70	8.86 ± 0.75	7.03 ± 0.55	6.10 ± 0.80
NU-14	8.70 ± 0.56	7.21 ± 0.70	6.10 ± 0.56	6.33 ± 0.21	6.23 ± 0.21	6.03 ± 0.60	7.10 ± 0.46	6.36 ± 0.64	6.23 ± 0.70
NU-15	9.10 ± 0.70	8.10 ± 0.60	7.13 ± 0.65	9.97 ± 0.55	8.26 ± 0.65	7.06 ± 0.75	7.13 ± 0.71	6.33 ± 0.65	6.03 ± 0.65
Clotr.			21.87 ± 1.07			22.56 ± 0.51			19.26 ± 0.56

Clotr. = clotrimazole

bacterial strains by disc diffusion method (13) at 70 µg/mL, 5 µg/mL and 30 µg/mL concentrations, respectively. A standard inoculum (1–2 × 10^7 c.f.u./mL 0.5 McFarland standards) was introduced onto the surface of sterile agar plates and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6 mm in diameter were prepared from Whatman no. 1 filter paper and sterilized by dry heat at 140°C for 1 h. The sterile discs previously soaked in a known concentrations of the test compounds were placed in nutrient agar medium. The plates were inverted and incubated for 24 h at 37°C. Vancomycin and amikacin were used as a standard drug. The inhibition zones (in mm) were measured and compared with the controls. All the tests were performed in triplicate and average reading was taken (Table 4).

Antifungal activity

Similarly, the newly prepared compounds were screened for their antifungal activity by paper disc method. For antifungal screening against *Aspergillus niger*, Czapek yeast extract agar was employed. Malt yeast agar with pH 7.0 was employed as culture media against *Candida albicans* and potato sucrose agar was used as culture medium against *Fusarium oxysporum* (14). All the tests were performed in triplicate and average reading was taken (Table 5).

RESULTS AND DISCUSSION

The compounds (**NU-1 to NU-15**) were synthesized according to Scheme 1. Ester (**1**) of 4-nitrobenzoic acid was synthesized using methanol and conc. sulfuric acid. In the second step, hydrazide (**2**) of this ester was prepared. This hydrazide was converted into potassium 3-(4-nitrobenzoyl) dithiocarbazate (**3**) using carbon disulfide and alcoholic potassium hydroxide which on cyclization formed 4-amino-3-mercapto-5-(4-nitro)phenyl-1,2,4-triazole (**4**).

Compound **4** reacted with chloroacetyl chloride in dioxane to form 4-chloroacetyl amino-3-mercapto-5-(4-nitro)phenyl-1,2,4-triazole (**5**)**.** In the last step, compound **5** was condensed with different secondary amines to form the final products (**NU-1 to NU-15**).

The physical parameters of all synthesized compounds are given in Table 2. Characterization of the synthesized compounds were carried out by determining their melting points, IR, ^{1}H NMR, ^{13}C NMR and MS spectra as well as elemental nitrogen analysis. All the final compounds showed C-N stretching band between 1320–1412 cm^{-1} and C=N stretching band between 1503–1588 cm^{-1}, which indicate the presence of 1,2,4-triazole ring. These compounds showed also absorption between 3000–3100 cm^{-1}, which is characteristics for aromatic C-H bonds. The absorption band between 1580–1619 cm^{-1} was a clear indication of aromatic C=C bonds. The presence of absorption band between 1610–1711 cm^{-1} and 3214–3421 cm^{-1} were a clear evidence for C=O stretching and N-H stretching of amide group, respectively. The presence of absorption band between 2510–2594 cm^{-1} reveals S-H stretching of free thio group. The absorption band near 1530 cm^{-1} shows N=O asymmetric stretching and around 1340 cm^{-1} N=O symmetric stretching of NO_2 group.

In ^{1}H-NMR spectra, a singlet of CONH was found in the range of ä 10.06–10.35 ppm and another singlet of thio group was observed in the range of δ 9.78–9.97 ppm. A singlet of $COCH_2$ was also found between δ 2.89–3.81 ppm. In ^{13}C- NMR spectra, C-3 and C-5 of the 1,2,4-triazole nucleus were observed in the range of δ 138–163 ppm. Carbonyl carbon and methylene carbon of –$NHCOCH_2N$< were found between δ 160–178 and δ 47–79 ppm, respectively. A solvent peak of DMSO-d_6 was observed at 44 ppm.

The compounds were evaluated for antimicrobial activity using disc diffusion method. A few of the compounds showed good activity comparing to standard drug. The data given in the Table include the size of filter paper disc (6 mm). The results of antibacterial activity showed that some appreciable inhibitions were shown by compounds **NU-2**, **NU-4** and **NU-11**. The most active compound was **NU-4**, which contain di-n-butylamino group attached to C_2 of acetamido group at 4-position of 1,2,4-triazole ring. Its zone of inhibition at 70 µg/mL concentration against *Staphylococcus aureus* (11 mm), *Bacillus subtilis* (11 mm), *Pseudomonas aeruginosa* (12 mm) and *Escherichia coli* (10 mm) were comparable to the standard drugs i.e., vancomycin (30 µg/mL) for Gram positive and amikacin (30 µg/mL) for Gram negative organisms. Among the bacterial strains taken for antibacterial activity, these three active compounds provided good response against *P. aeruginosa*. The results of antifungal activity showed that some appreciable inhibition were shown by **NU-2**, **NU-4** and **NU-12**. Out of these, the inhibition shown by compound **NU-4** was the best. Its zone of inhibition at 70 µg/mL concentration against *Candida albicans* (11 mm), *Aspergillus niger* (14 mm) and *Fusarium oxysporum* (10 mm) could be compared with the standard drug i.e., clotrimazole (30 µg/mL) and the most appropriate response was against *Aspergillus niger.*

The antifungal activity of synthesized compounds against fungal strains were as follows: *Aspergillus niger* > *Candida albicans* > *Fusarium oxysporum*.

Thus, it can be said that compound **NU-4** showed not only good antibacterial activity but was also good antifungal agent. SAR study of the synthesized compounds showed that dialkylamine substitution is preferable at position C_2 of acetamido group at 4-position of 1,2,4-triazole ring than heterocyclic amine (i.e., morpholine).

CONCLUSION

It can be concluded that substituted 1,2,4-triazole moiety displays not only good antifungal activity but also provides good antibacterial activity as well. Alkylamine substitution is better than heterocyclic amine on 1,2,4-triazole moiety. These compounds can be considered as lead molecules for future investigations.

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REFERENCES

1. The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, 12th edn., p. 1634, Merck & Co. Inc., Whitehouse station, N.J 1996.
2. Rao G. K., Rajasekaran S., Attimarad M.: Indian J. Pharm. Sci. 62, 475 (2000).
3. Ozkirimli S., Idli Apak T., Kiraz M., Yegenoglu Y.: Arch. Pharm. Res. 28, 1213 (2005).
4. Mir I., Siddiqui M.T.: Tetrahedron 26, 5235 (1970).
5. Holla B.S., Veerendra B., Shivananda M.K., Poojary B.: Eur. J. Med. Chem. 38, 759 (2003).
6. Al-soud Y.A., Al-Masoud N.A., Ferwanah A.E.: Bioorg. Med. Chem. 11, 1701 (2003).
7. Boschelli D.H., Connor D.T., Bornemeier D.A., Dyer R.D., Kennedy J.A., et al.: J. Med. Chem. 36, 1802 (1993).
8. Parmar S.S., Chaudhary M., Chaudhary A.K., Kumar S., Spiro P.R.: J. Pharm. Sci. 66, 971 (1977).
9. Gall M., Lathi R.A., Rudzik A.D., Duchamp D.J., Chidester C., Scahill T.: J. Med. Chem. 21, 542 (1978).
10. Kane J.M., Dudley M.W., Sorensen S.M., Miller F.P.: J. Med. Chem. 31, 1253 (1988).
11. Mhasalkar M.Y., Shah M.H., Nikam S.T., Anantanarayanan K.G., Deliwala C.V.: J. Med. Chem. 13, 672 (1970).
12. Romine J.L., Martin S.W., Gribkoff V.K., Boissard C.G., Dworetzky S.I., Natale J., et al.: J. Med. Chem. 45, 2942 (2002).
13. Cruickshank R., Duguid J.P., Marmion B.P., Swain R.H.A.: Medicinal Microbiology, 12th edn. vol. II, p. 196, Churchill Livingstone, London 1975.
14. Microbial Type Culture Collection and Gene Bank (MTCC), 5th edn., p. 130, 137 and 138, (Medium No-137), Institute of Microbial Technology (IMTECH), Chandigarh 2000.

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