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A BASE CATALYZED, AN EFFICIENT SYNTHESIS OF 3,5 DISUBSTITUTED-(NH)-1,2,4-TRIAZOLES FROM S-METHYL ISOTHIOAMIDE AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

A convenient synthesis of 3, 5-disubstituted-(NH)-1, 2, 4-triazoles was carried out from S-methyl isothioamide and substituted phenyl hydrazide using different bases with high yield. The newly synthesised compound was characterised by IR, NMR and Mass spectroscopy. All the synthesised compounds were evaluated for their antimicrobial activity.

Keywords: 1,2,4-triazole, S-Methyl Isothiomide, Hydrazide, Antibacterial Activity, Antifungal Activity.

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INTRODUCTION

Triazoles are well-known heterocyclic compounds, found in many potent biologically active molecules. The biological activity of substituted triazoles has made them a focus of medicinal chemistry over the years. Triazoles have potent antimicrobial activity[1-3] and anticancer activity.[4,5] Some of the 3,5-disubstituted-(NH)-1,2,4-triazoles are used as xanthine oxidoreductase inhibitors in the treatment of hyperuricemia.[6,7] Several procedures for the synthesis of triazoles have been extensively studied.[8-12] Microwave-assisted synthesis of triazoles from S-methyl isothioamide and hydrazide, and also from imidates with hydrazide, is reported.[13,14] Direct synthesis of triazoles was also carried out from amidines and nitriles using copper as a catalyst.[15] Nowadays, a great deal has been focused on the modification of reaction conditions to afford higher yields, lowering the reaction time and carrying out an easy workup procedure. Herein, we have developed a simple procedure for the synthesis of 1,2,4-triazoles carrying an amide linker with excellent yield.

EXPERIMENITAL

Material and Methods

5-amino-2-fluorobenzonitrile was used as received, while substituted acyl hydrazides were synthesised from the corresponding ester of aryl acid and hydrazine hydrate. Thin-layer chromatography using silica gel G (E. Merck) plates was used to assess the reactions. NMR spectra were recorded using a Bruker 400-MHz instrument, and IR spectra were recorded on a Shimadzu FTIR-8400 spectrophotometer.

General Procedure

Synthesis of N-(3-cyano-4-fluorophenyl)-4-fluorobenzamide (3)

Thionyl chloride (5.34 mL, 0.0734 mol) was added to a solution of 4-fluorobenzoic acid (1) (5.15 g, 0.0367 mol) in dry DCM (50 mL). Upon the addition of 1-2 drops of DMF, a mixture was heated at reflux for 1h. The resultant solution was then cooled to 25oC, to this solution a mixture of 5-amino-2-fluorobenzonitrile (2) (5 g, 0.0367 mol) in DCM (30 mL) was added dropwise with stirring. The reaction mixture was refluxed for 2 h. After the completion of the reaction, a mixture was washed with water followed by saturated $NaHCO_3$ solution. The organic phase was dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure to afford an analytically pure product. Yield 9 g (95%); m.p. 126-128^oC °C; Anal. Calcd for: $C_{14}H_8F_2N_2O$: C, 65.12; H, 3.12; N, 10.85; Found: C, 65.32; H, 3.18; N,

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10.77; IR (KBr): 3298, 3144, 3082, 2233, 1654, 1602, 1552, 1502, 1408, 1327, 1226, 1163, 1014, 823, 773, 675, 545, 493 cm^{-1}; ^1H NMR: δ 7.385-7.474 (m, 2H, Ar-H), 7.537-7.583 (t, 1H, Ar-H), 8.028-8.081 (m, 3H, Ar-H), 8.263-8.284 (dd, 1H, Ar-H, j=2.4,5.6 Hz), 10.611 (s, 1H, -NH amide); MS (m/z): 250 (M^+).

Synthesis of N-(3-carbamothioyl-4-fluorophenyl)-4-fluorobenzamide (4)

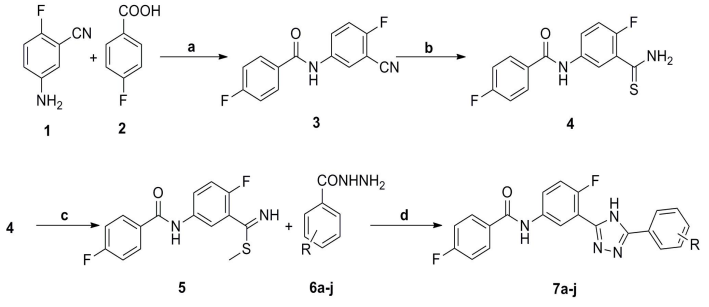
To the stirred solution of N-(3-cyano-4-fluorophenyl)-4-fluorobenzamide (3) (2 g, 7.745 mmol) in DMF (20 mL), $MgCl_2{\cdot}6H_2O$ (3.93 g, 19.363 mmol) was added at 0- 5 °C. The resultant solution was stirred for 10 min, then sodium hydrogensulphide (0.868 g, 15.490 mmol) was added portion-wise while cooling. A mixture was stirred at room temperature for 1 h. After the completion of the reaction, the mixture was poured into cold water. The separated product was filtered and washed with water, and then dried in an oven. Yield 2.2 g (97%); m.p. 156-158°C °C; Anal. Calcd for: $C_{14}H_8F_2N_2O$: C, 65.12; H, 3.12; N, 10.85; Found: C, 65.21; H, 3.20; N, 10.91; IR (KBr): 3504, 3450, 3358, 3281, 3174, 3107, 1658, 1568, 1431, 1377, 1215, 1169, 1093, 835, 740, 605, 522 cm^{-1}; ^1H NMR: *δ* 7.221-7.268 (t, 1H, Ar-H),7.361-7.405 (t, 2H, Ar-H), 7.849-7.878 (dd, 3H, Ar-H), 8.011-10.8.072 (dd, 1H, Ar-H), 9.644 (s, 1H, $-NH_2$), 10.208 (s, 1H, $-NH_2$), 10.422 (s, 1H, -NH amide); MS (*m/z*): 292 (M^+).

Synthesis of Methyl -2-fluoro-5-(4-fluorobenzamido) benzimidothioate (5)

To a solution of N-(3-carbamothioyl-4-fluorophenyl)-4-fluorobenzamide (4) (2 g, 6.842 mmol) in diethyl ether (20 mL) was added methyl iodide (0.51 mL, 8.210 mmol) while stirring at 0 °C. This solution was stirred for 30 min at 0oC then warmed to room temperature for 10 h. The solid obtained was filtered and washed with diethyl ether (20 mL), pure product was obtained. Yield 1.9 g (90%); m.p. 168-170; Anal. Calcd for: $C_{15}H_{12}F_2N_2OS$: C, 58.81; H, 3.95; N, 9.14; Found: C, 58.97; H, 3.79; N, 9.21IR (KBr): 3369, 3269, 3076, 1649, 1604, 1531, 1423, 1352, 1246, 1161, 1095, 889, 759, 685, 551 cm^{-1}; ^1H NMR: δ 2.860 (s, 3H, S-Me), 7.395-7.439 (t, 2H, Ar-H), 7.527-7.574 (t, 1H, Ar-H), 7.910-8.075 (m, 3H, Ar-H), 8.270-8.291 (dd, 1H, Ar-H), 11.978 (s, 1H, =NH), 10.681 (s, 1H, -NH amide); MS (m/z): 306 (M^+).

General Synthesis of 3,5-disubstituted-1,2,4-triazoles (7a-j).

To a mixture of methyl-2-fluoro-5- (4-fluorobenzamido) benzimidothioate **(5)** (3.26 mmol) and hydrazides **(6a-j)** (3.26 mmol) in 10 mL DMF was added K2CO3 (9.8 mmol) at room temperature. The resulting reaction mixture was further stirred at rt for 30 min, then heated up to 120oC for 3-4 h. After completion of the reaction, the mixture was poured into ice-cold water. The obtained solid was filtered, washed with water and dried in an oven.



Scheme 1. Reagents and conditions: (a) DCM,$SOCl_2$, reflux (b) $MgCl_2$, NaSH, DMF, rt (c) MeI, diethyl ether, rt (d) base, DMF, 120°C.

Scheme-1: Synthesis of 3,5-disubstituted-(NH)-1,2,4-triazoles

Spectral Characterizations

4-fluoro-N-(4-fluoro-3-(5-(4-(trifluoromethyl)phenyl)-4H-1,2,4-triazol-3-yl)phenyl)benzamide (7a)

Yield: 95%; m.p. 228-230 °C; Anal. Calcd for $C_{22}H_{13}F_5N_4O$: C, 59.46; H, 2.95; N, 12.61; Found: C, 59.52; H, 2.87; N, 12.68; IR (KBr, cm^{-1}): 3173, 3082, 2943, 1921, 1737, 1656, 1543, 1367, 1219, 1168, 1066, 848, 763, 682, 588 cm^{-1}; ^1H NMR: δ 7.387-7.431 (t, 3H, Ar-H), 7.923-7.997 (m, 2H, Ar-H), 8.059-8.178 (m, 3H, Ar-H), 8.301-8.321 (d, 2H, Ar-H, j=8 Hz), 8.570-8.580 (d, 1H, Ar-H, j=4 Hz), 10.553 (s, 1H, -NH amide), 14.684 (s, 1H, -NH triazole); 13C NMR (100 MHz, DMSO): 115.28, 115.56, 116.32,

123.17, 124.46, 126.48, 128.38, 129.21, 129.82, 129.93, 130.68, 131.42, 135.44, 152.34, 156.67, 157.62, 162.46, 164.58; MS (m/z): 444 (M^+).

Table-1: Physical Data of 3,5-disubstituted Triazoles(7a-j)

Entry	R	Time h	Yield %	mp ºC
7a	4-CF_3 C_6H_4	3.5	95	228-230
7b	4-Br C_6H_4	3.2	94	204-206
7c	4-F C_6H_4	3.0	88	190-192
7d	CH2-(2-Cl C_6H_4)	2.5	92	182-184
7e	CH2-(3-Cl C_6H_4)	2.5	91	188-190
7f	CH2-(3,4-di-Cl C_6H_3)	2.7	98	194-196
7g	2,4-di-Cl C_6H_3	3.5	96	236-238
7h	2,6-di-Cl C_6H_3	3.5	87	230-232
7i	4-substituted-5-Me-isoxazole	4.0	65	222-224
7j	4-$NO_2C_6H_4$	4.5	72	232-234

N-(3-(5-(4-bromophenyl)-4H-1,2,4-triazol-3-yl)-4-fluorophenyl)-4-fluorobenzamide (7b)
Yield: 94%; m.p. 204-206 °C; Anal. Calcd for $C_{21}H_{13}BrF_2N_4O$: C, 55.40; H, 2.88; N, 12.31; Found: C, 55.37; H, 2.94; N, 12.35; IR (KBr, cm^{-1}): 3163, 3082, 2942, 1941, 1751, 1658, 1526, 1381, 1214, 1167, 1061, 862, 764, 687, 585 cm^{-1}. ^1H NMR: δ 7.498-7.564 (t, 5H, Ar-H),), 7.998 (dd, 1H, Ar-H, j=3.4, 8.8 Hz), 8.123-8.156 (m, 4H, Ar-H), 8.651 (dd, 1H, Ar-H, j=2.6, 6.8 Hz), 10.532 (s, 1H, -NH amide), 14.578 (s, 1H, -NH triazole); ^{13}C NMR (100 MHz, DMSO): 114.54, 115.38, 118.46, 125.86, 127.36, 129.45, 131.28, 132.12, 132.45, 133.45, 133.58, 136.87, 154.68, 157.18, 159.78, 163.56, 164.72; MS (m/z): 455 (M^+).

4-fluoro-N-(4-fluoro-3-(5-(4-fluorophenyl)-4H-1,2,4-triazol-3-yl)phenyl)benzamide (7c)
Yield: 88 %; m.p. 190-192 °C; Anal. Calcd for $C_{21}H_{13}F_3N_4O$: C, 63.96; H, 3.32; N, 14.21; Found: C, 63.83; H, 3.41; N, 14.27; IR (KBr, cm^{-1}): 3156, 3078, 2952, 1934, 1740, 1661, 1532, 1375, 1208, 1173, 1052, 851, 773, 693, 579 cm^{-1};^1H NMR: δ 7.377-7.438 (m, 5H, Ar-H), 7.970 (dd, 1H, Ar-H, j=3.6, 8.6 Hz), 8.079-8.146 (m, 4H, Ar-H), 8.535 (dd, 1H, Ar-H, j=2.8, 6.4 Hz), 10.517 (s, 1H, -NH amide), 14.542 (s, 1H, -NH triazole); ^{13}C NMR (100 MHz, DMSO): 115.12, 116.23, 117.18, 118.42, 122.74, 124.36, 126.48, 128.36, 129.30, 130.18, 132.38, 132.67, 157.46, 158.18, 161.48, 164.17, 166.47; MS (m/z): 394 (M^+).

N-(3-(5-(2-chlorobenzyl)-4H-1,2,4-triazol-3-yl)-4-fluorophenyl)-4-fluorobenzamide (7d)
Yield: 92 %; m.p. 182-184; Anal. Calcd $C_{22}H_{15}ClF_2N_4O$: C, 62.20; H, 3.56; N, 13.19; Found: C, 62.31; H, 3.51; N, 13.21; IR (KBr, cm^{-1}): 3261, 3174, 3066, 2918, 1645, 1545, 1498, 1373, 1230, 1157, 1093, 848, 750, 680, 561 cm^{-1}; ^1H NMR: δ 4.262 (s, 2H, -CH_2), 7.342-7.402 (m, 6H, Ar-H), 7.482 (d, 1H, Ar-H, j=5.6 Hz), 7.949 (s, 1H, Ar-H), 8.071 (s, 1H, Ar-H), 8.366 (s, 1H, Ar-H), 10.451 (s, 1H, -NH amide); ^{13}C NMR (100 MHz, DMSO): 32.46, 115.68, 115.82, 116.17, 117.46, 121.72, 122.98, 126.12, 126.43, 127.19, 128.92, 130.49, 132.26, 132.88, 134.62, 138.48, 154.36, 164.42, 166.38; MS (m/z): 424 (M^+).

N-(3-(5-(3-chlorobenzyl)-4H-1,2,4-triazol-3-yl)-4-fluorophenyl)-4-fluorobenzamide (7e)
Yield: 91 %; m.p. 188-190; Anal. Calcd $C_{22}H_{15}ClF_2N_4O$: C, 62.20; H, 3.56; N, 13.19; Found: C, 62.33; H, 3.54; N, 13.22; IR (KBr, cm^{-1}): 3251, 3192, 3070, 2941, 1901, 1734, 1660, 1500, 1371, 1271, 1163, 1093, 840, 769, 682, 511 cm^{-1}; ^1H NMR: δ 4.262 (s, 2H, -CH2), 7.342-7.402 (m, 6H, Ar-H), 7.482 (d, 1H, Ar-H, j=5.6 Hz), 7.949 (s, 1H, Ar-H), 8.071 (s, 1H, Ar-H), 8.366 (s, 1H, Ar-H), 10.451 (s, 1H, -NH amide); ^{13}C NMR (100 MHz, DMSO): 32.52, 115.42, 116.92, 116.46, 117.93, 121.71, 122.68, 126.17, 126.58, 127.32, 128.93, 130.67, 132.27, 132.89, 134.68, 138.49, 154.37, 163.96, 166.72; MS (m/z): 424 (M^+).

N-(3-(5-(3,4-dichlorobenzyl)-4H-1,2,4-triazol-3-yl)-4-fluorophenyl)-4-fluorobenzamide(7f) Yield: 98 %; m.p. 194-196ºC; Anal. Calcd $C_{22}H_{14}Cl_2F_2N_4O$: C, 57.53; H, 3.07; N, 12.20; Found: C, 57.48; H, 3.10; N, 12.18; IR (KBr, cm^{-1}): 3250, 3173, 3059, 2910, 1651, 1602, 1543, 1379, 1238, 1135, 1043, 889, 771, 675, 555 cm^{-1}; ^1H NMR: δ 4.183 (s, 2H, -CH2), 7.232-7.407 (m, 4H, Ar-H), 7.481-7.631 (m, 2H, Ar-

H), 7.930 (s, 1H, Ar-H), 8.073 (dd, 2H, Ar-H, j=5.6, 8.8 Hz), 8.411 (d, 1H, Ar-H, j=4 Hz), 10.450 (s, 1H, -NH amide); ^{13}C NMR (100 MHz, DMSO): 31.72, 115.32, 116.47, 116.52, 117.48, 121.72, 122.69, 126.27, 126.52, 126.98, 129.32, 131.12, 133.46, 133.92, 134.96, 138.87, 155.12, 164.43, 166.89; MS (m/z): 459 (M^+).

N-(3-(5-(2,4-dichlorophenyl)-4H-1,2,4-triazol-3-yl)-4-fluorophenyl)-4-fluorobenzamide(7g) Yield: 96 %; m.p. 236-238^{o}C; Anal. Calcd $C_{22}H_{14}Cl_2F_2N_4O$: C, 57.53; H, 3.07; N, 12.20; Found: C, 57.49; H, 3.09; N, 12.26; IR (KBr, cm^{-1}): 3272, 3164, 3072, 2924, 1651, 1539, 1484, 1367, 1237, 1162, 1079, 854, 761, 686, 587 cm^{-1}; ^{1}H NMR: δ 7.399 (t, 2H, Ar-H), 7.489 (t, 1H, Ar-H), 7.613 (t, 1H, Ar-H), 7.802 (s, 1H, Ar-H), 7.909 (s, 1H, Ar-H), 8.025 (d, 1H, Ar-H), 8.089 (dd, 2H, Ar-H, j=5.6, 8 Hz), 8.538 (s, 1H, Ar-H), 10.555 (s, 1H, -NH amide), 14.622 (s, 1H, -NH triazole); ^{13}C NMR (100 MHz, DMSO): 115.32, 115.62, 116.68, 122.48, 123.42, 126.54, 127.72, 128.48, 129.13, 130.42, 130.98, 131.17, 131.37, 133.62, 134.76, 136.48, 164.12, 166.17; MS (m/z): 445 (M^+).

N-(3-(5-(2,6-dichlorophenyl)-1H-1,2,4-triazol-3-yl)-4-fluorophenyl)-4-fluorobenzamide (7h*)*
Yield: 87 %; mp 230-232˚C; Anal. Calcd $C_{21}H_{12}Cl_2F_2N_4O$: C, 56.65; H, 2.72; N, 12.58; Found: C, 56.36; H, 2.92; N, 12.38; IR (KBr, cm^{-1}): 3276, 3154, 3040, 2943, 1675, 1580, 1490, 1323, 1297, 1122, 1026, 850, 765, 685, 580 cm^{-1};^{1}H NMR: δ 7.307 (t, 2H, Ar-H), 7.472 (t, 1H, Ar-H), 7.712-7.734 (d, 2H, Ar-H, j=8.8 Hz), 7.854-7.876 (d, 2H, Ar-H, j=8.8 Hz), 7.912 (s, 1H, Ar-H), 8.017 (t, 1H, Ar-H), 8.645 (s, 1H, Ar-H), 10.542 (s, 1H, -NH amide), 14.572 (s, 1H, -NH triazole); ^{13}C NMR (100 MHz, DMSO): 115.45, 115.732, 117.46, 121.39, 123.68, 126.49, 127.91, 128.17, 129.64, 130.32, 131.15, 131.62, 132.36, 133.87, 134.56, 137.68, 164.72, 166.86; MS (*m/z*): 445 (M^+).

4-fluoro-N-(4-fluoro-3-(5-(5-methylisoxazol-4-yl)-4H-1,2,4-triazol-3-yl)phenyl)benzamide(7i)
Yield: 65 %; m.p. 222-224; Anal. Calcd $C_{19}H_{13}F_2N_5O_2$: C, 59.84; H, 3.44; N, 18.37; Found: C, 59.91; H, 3.38; N, 18.41; IR (KBr, cm^{-1}): 3259, 3181, 3067, 2925, 1643, 1609, 1538, 1383, 1242, 1141, 1052, 877, 763, 681, 562 cm^{-1}; ^{1}H NMR: δ 2.892 (s, 3H, -CH3), 7.418- 7.431 (m, 2H, Ar-H), 7.554 (t, 1H, Ar-H), 7.957 (s, 1H, Ar-H), 8.063-8.100 (m, 2H, Ar-H), 8.265-8.287 (dd, 1H, Ar-H, j=2.8, 6 Hz), 8.447-8.471 (dd, 1H, Ar-H, j=2.8, 6.8 Hz), 10.616 (s, 1H, -NH amide); ^{13}C NMR (100 MHz, DMSO): 15.31, 110.12, 115.62, 116.48, 122.32, 123.37, 125.41, 128.12, 129.44, 132.13, 152.28, 153.98, 156.94, 162.37, 164.32, 167.12; MS (m/z): 381 (M^+).

4-fluoro-N-(4-fluoro-3-(5-(4-nitrophenyl)-4H-1,2,4-triazol-3-yl)phenyl)benzamide (7j)
Yield: 72 %; m.p. 232-234; Anal. Calcd for $C_{21}H_{13}F_2N_5O_3$: C, 59.86; H, 3.11; N, 16.62; Found: C, 59.71; H, 3.17; N, 16.57; IR (KBr, cm^{-1}): 3262, 3187, 3062, 2937, 1922, 1746, 1674, 1512, 1368, 1269, 1174, 1087, 853, 776, 691, 523 cm^{-1}; ^{1}H NMR: δ 7.428 (t, 3H, Ar-H), 7.856 (m, 2H, Ar-H), 8.124 (m, 3H, Ar-H), 8.265-8.287 (d, 2H, Ar-H, j=8.8 Hz), 7.909 (s, 1H, Ar-H), 8.025 (d, 1H, Ar-H), 8.089 (dd, 2H, Ar-H, j=5.6, 8 Hz), 8.538 (s, 1H, Ar-H), 10.555 (s, 1H, -NH amide), 14.622 (s, 1H, -NH triazole); ^{13}C NMR (100 MHz, DMSO): 116.37, 117.32, 120.44, 122.68, 123.78, 124.92, 126.94, 128.12, 129.19, 130.47, 130.92, 132.44, 138.68, 149.42, 158.19, 163.12, 166.98; MS (m/z): 421 (M^+).

RESULTS AND DISCUSSION

Synthesis of 1,2,4-triazoles by most reported procedures involves a long reaction time and results in a lower yield. S-methyl isothioamide is a very useful precursor in the synthesis of various heterocycles. Generally, S-methyl isothioamide was synthesised by methylation of thioamide derivatives, which can be produced by corresponding nitriles by reported methods [16]We have synthesised 3,5-disubstituted-(NH)-1,2,4-triazoles by the condensation of S-methyl isothioamide with acyl hydrazide in the presence of various bases. To achieve great yields, the condensation of **5** with **6a-j** to produce **7a-j** was investigated using a variety of bases in different solvents (Table-1). We get more than 90% yield and easy to work up, which is better than most of the previously reported methods. All the synthesised compounds were screened against a variety of bacterial strains (Table-2) such as *E. coli*, *S.pyogenus*, *S. aureus*, *P. aeruginosa* and fungi strains (Table-3) *C. albicans, A.niger, and A.c lavatus* at minimal inhibitory concentration (MIC). Standard drugs like Ampicillin, Chloramphenicol, Nystatin and Greseofulvin were used for comparison purposes.

Table-2: Antibacterial Activity of Compounds 7a-j:

S.No.	Code no.	MIC (µg/mL)			
		E.coli	*P.aeruginosa*	*S.aureus*	*S.pyogenus*
1	7a	250	500	100	500
2	7b	250	250	250	500
3	7c	250	500	200	500
4	7d	100	250	250	250
5	7e	500	200	500	500
6	7f	500	250	250	250
7	7g	250	100	250	500
8	7h	250	250	500	500
9	7i	100	250	250	500
10	7j	250	250	500	125
Ampicillin		100	100	250	100
Chloramphenicol		50	50	50	50

Table-3: Antifungal Activity of Compounds 7a-j:

S. No.	Code No.	MIC (µg/mL)		
		C.albicans	*A.niger*	*A.clavatus*
1	7a	500	1000	1000
2	7b	100	200	200
3	7c	200	500	500
4	7d	250	250	500
5	7e	500	1000	1000
6	7f	200	500	>1000
7	7g	250	100	500
8	7h	500	200	500
9	7i	200	200	500
10	7j	>1000	500	>1000
Nystatin		100	100	100
Greseofulvin		500	100	100

CONCLUSION

In summary, we have synthesised 1,2,4-triazole derivatives using s-methyl thioamide and acyl hydrazide by simply heating with base in excellent yield and purity. All the synthesised compounds were evaluated for their antimicrobial activity. The investigation of antimicrobial and antifungal screening data revealed that all the tested compounds **7a-j** showed moderate to significant activity.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Helth and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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