SYNTHESIS AND *In vitro* ANTIMICROBIAL ACTIVITY OF SOME TRIAZOLE DERIVATIVESRAVINESH MISHRA*, RAJIV KUMAR, SURESH KUMAR, JASEELA MAJEED,
MOHD RASHID, SAMEER SHARMA

*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110 062, India.

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ABSTRACT

Some 4-[(1-(substituted)methylidene)-amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazol (3a-3f) and N-[5-(4-substituted)-1H-1,2,3-triazol-1-yl]isonicotinamide derivatives (5a-5e) were synthesized by a sequence of reactions starting from isonicotinic acid hydrazide and is illustrated in scheme 1 and 2. The antibacterial and antifungal activities of newly synthesized compounds were tested by the disc diffusion method using nutrient agar medium against various microorganisms such as gram positive *Staphylococcus aureus* and *Bacillus subtilis*, gram negative *Escherichia coli* and the fungi *Aspergillus niger* and *Candida albicans*. Ciprofloxacin and Fluconazole at 50 µg/mL were used as standard drugs for antibacterial and antifungal activities, respectively. All the synthesized compounds showed significant activity against various microorganisms.

Key words: Triazole derivatives, Antimicrobial activity, Ciprofloxacin, Fluconazole.

INTRODUCTION

Since the past few decades, the literature has been enriched with progressive findings about the synthesis and pharmacological activities of various substituted azole derivatives. It reveals that 1,2,4 and 1,2,3-triazoles possess broad spectrum of activities such as antimicrobial¹⁻⁴, anti-inflammatory⁵, analgesic⁶, antitumoral⁷, antihypertensives⁸, anticonvulsant and antiviral⁹. Some of the antimicrobial triazole derivatives used as common antibiotics such as Amphotericin B possess toxic effect on humans along with their antimicrobial effects¹⁰. Although there are antimicrobial agents having different structures are frequently used in the treatment of fungal infections, so there is increasing resistance to these drugs. To overcome the development of drug resistance, it is necessary to synthesize a new class of antimicrobial compounds possessing different chemical properties from those that are used commonly. 5-Thioxo-1,2,4-triazole containing a substituted aromatic aldehyde side chain is a ideal heterocyclic for antifungal activity. The following antifungal 1,2,4-triazole derivatives are applicable in medicine like Fluconazole, Itraconazole and Terconazole¹¹.

EXPERIMENTAL

The chemical reactives were supplied by E. Merck (Germany) and S.D Fine chemicals (India). Melting points were determined by open tube capillary method and were uncorrected. The purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in toluene-ethyl formate-formic acid (5:4:1) and benzene-methanol (8:2) solvent systems, the spots were located under iodine vapors and UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr pellets). ¹H-NMR spectra were recorded by a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO-d₆/CDCl₃ and mass spectra under electron impact conditions (EI) were recorded at 70 eV ionizing voltage with a VG Prospec instrument and are presented as m/z. UV spectra were recorded on a UV-Visible Spectrophotometer Pharma Spec-1700 (SHIMADZU).

Synthesis of 4-amino-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazol (2)

Isonicotinic acid hydrazide 13.7 g (0.1 mol) was dissolved in 200 mL absolute alcohol containing potassium hydroxide 11.2 g (0.1 mol) at room temperature 12.5 mL carbondisulfide was added in parts and was stirred for 16 hours at room temperature. 100 mL of diethyl ether was added and stirred for further 3 h. 10.3 g (0.1 mol, 99%) hydrazine hydrate was added gradually to the potassium dithiocarbazinate salt dissolved in 100 mL water with stirring and was refluxed for 8 h during which hydrogen sulphide gas evolved and the colour of the reaction mixture changed to deep green. It was then cooled and acidified with hydrochloric acid to pH 1. The yellow colored solid was isolated by filtration and recrystallised from ethanol to give compound (2)¹².

General procedure for synthesis of 4-[(1-(substituted)methylidene)-amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazol derivatives (3a-3f)

Few drops of glacial acetic acid were added to a solution of 0.01 mol of compound 2 (4-amino-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazol) in dimethylformamide (20 mL) and 0.01 mol of various benzaldehyde derivatives was added and refluxed for 9 hours. The reaction mixture was cooled and the pre-

cipitate obtained was filtered, dried in vacuum and recrystallised from ethanol to give compound 3a-3f. The compounds were synthesized as illustrated in scheme 1¹³.

Scheme 1. 4-[(1-(substituted)methylidene)-amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazol derivatives (3a-e).

General procedure for synthesis of N-[5-(4-substituted)-1H-1,2,3-triazol-1-yl]isonicotinamide derivatives (5a-5e)

Isonicotinic acid hydrazide 1.37 g (0.01 mol) was dissolved in 30 mL ethanol and reacted with various equimolar substituted benzaldehyde derivatives were added in presence of few drops of glacial acetic acid and refluxed for 5 h. Then it was cooled, filtered and recrystallised from ethanol. This gave an intermediate hydrazone compound, which was cyclised by passing freshly prepared diazomethane gas to the dissolved compound in 1,4 dioxane. The solution was allowed to stand for 24 h so that complete cyclisation took place, the product obtained after cooling was recrystallised from 1,4 dioxane to yield compound (5a-5e). The compounds were synthesized as is shown in scheme 2^{14,15}.

Scheme 2. N-[5-(4-substituted)-1H-1,2,3-triazol-1-yl]isonicotinamide derivatives (5a-e).

Data Analysis

4-[[1-(4'-chlorophenyl)methylidene]amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole, (3a), colourless solid, yield 44%, mp 276-278°C, R_f 0.76, UV $\lambda_{\max}$ (DMF) 245.8 nm; FTIR (KBr) ν cm⁻¹ 3013 (Ar C-H stretching), 2563 (S-H stretching), 1603, 1485 (Ar C=C stretching), 1563 (C=N stretching), 1313 (C-N stretching), 828 (C-Cl stretching), 688 (C-S stretching); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 6.06 (s, 1H, N=CH), 7.59-8.76 (m, 8H, aromatic protons), 12.59 (s, 1H, S-H). Anal. calcd. for C₁₄H₁₀N₅SCl: C, 54.30; H, 4.25; N, 21.11. Found: C, 54.34; H, 4.21; N, 21.09. FABMS (m/z) 314(M⁺), 315 (M⁺+1). Mol. Wt.: 315.

4-[[1-(4'-dimethylaminophenyl)methylidene]amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole, (3b), colourless solid, yield 31%, mp 224-226°C, R_f 0.82, UV $\lambda_{\max}$ (DMF) 260.7 nm; FTIR (KBr) ν cm⁻¹ 3132 (Ar C-H stretching), 2544 (S-H stretching), 1523 (C=N stretching), 1344 (C-N stretching), 671 cm⁻¹ (C-S stretching); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 3.20 [s, 6H, N(CH₃)₂], 8.08 (s, 1H, N=CH), 7.59-8.78 (m, 8H, aromatic protons), 12.32 (s, 1H, SH). Anal. calcd. for C₁₅H₁₆N₆S: C, 57.67; H, 5.16; N, 26.90. Found: C, 57.64; H, 5.18; N, 26.93. FABMS (m/z) 323 (M⁺), 324(M⁺+1). Mol. Wt.: 324.

4-[[1-(3'-nitrophenyl)methylidene]amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole, (3c), colourless solid, yield 66%, mp 288-290°C, R_f 0.79, UV $\lambda_{\max}$ (DMF) 221.9 nm; FTIR (KBr) ν cm⁻¹ 3033 (Ar C-H stretching), 2579 (S-H stretching), 1609, 1585, 1452 (Ar C=C stretching), 1520 (C=N stretching), 1353 (Ar-NO₂), 1315 (C-N stretching), 698 cm⁻¹ (C-S stretching); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 5.58 (s, 1H, N=CH), 7.78-8.71 (m, 8H, aromatic protons), 14.02 (s, 1H, S-H). Anal. calcd. for C₁₄H₁₀N₆SO₂: C, 51.53; H, 3.09; N, 25.02. Found: C, 51.52; H, 3.12; N, 25.14. FABMS (m/z) 325(M⁺), 326(M⁺+1). Mol. Wt.: 326.

4-[[1-(3'-hydroxyphenyl)methylidene]amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole, (3d), colourless solid, yield 62%, mp 282-284°C, R_f 0.66, UV $\lambda_{\max}$ (DMF) 234.6 nm; FTIR (KBr) ν cm⁻¹ 3450 (O-H stretching), 3032 (Ar C-H stretching), 2579 (S-H stretching), 1609, 1585, 1453 (Ar C=C stretching), 1546 (C=N stretching), 1315 (C-N stretching), 699 (C-S stretching); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 5.64 (s, 1H, N=CH), 7.82 (s, 1H, O-H), 7.86-8.72 (m, 8H, aromatic protons), 13.96 (s, 1H, S-H). Anal. calcd. for C₁₄H₁₁N₅SO: C, 56.55; H, 3.73; N, 23.10. Found: C, 56.58; H, 3.76; N, 23.13. FABMS (m/z) 296(M⁺), 297(M⁺+1). Mol. Wt.: 297.

4-[[1-(4'-methoxyphenyl)methylidene]amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole, (3e), colourless solid, yield 50.8%, mp 272-274°C, R_f 0.72, UV $\lambda_{\max}$ (DMF) 212.4 nm; FTIR (KBr) ν cm⁻¹ 3051 (Ar C-H stretching), 2872 (C-O-CH₃), 2621 (S-H stretching), 1606 (Ar C=C stretching), 1541 (C=N stretching), 1377 (C-N stretching), 678 (C-S stretching); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 3.79 (s, 3H, -O-CH₃), 7.67 (s, 1H, N=CH), 7.25-7.80 (m, 8H, aromatic protons), 11.02 (s, 1H, SH). Anal. calcd. for C₁₅H₁₃N₅SO: C, 57.86; H, 4.21; N, 22.49. Found: C, 57.84; H, 4.22; N, 22.46. FABMS (m/z) 310 (M⁺), 311 (M⁺+1). Mol. Wt.: 311.

4-[[1-(3'-chlorophenyl)methylidene]amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazole, (3f), colourless solid, yield 51%, mp 296-298°C, R_f 0.81, UV $\lambda_{\max}$ (DMF) 240.6 nm; FTIR (KBr) ν cm⁻¹ 2975 (Ar C-H stretching), 2648 (S-H stretching), 1664 (Ar C=C), 1523 (C=N stretching), 1365 (C-N stretching), 815 (C-Cl stretching), 682 (C-S stretching). ¹H NMR (400 MHz DMSO-d₆ δ ppm) 5.69 (s, 1H, N=CH), 7.72-7.90 (m, 4H, Aromatic protons), 8.01-8.13 (m, 4H, aromatic protons), 12.49 (s, 1H, S-H). Anal. calcd. for C₁₄H₁₀N₅SCl: C, 53.25; H, 3.19; N, 22.18. Found: C, 53.29; H, 3.17; N, 22.20. FABMS (m/z) 314(M⁺+1), 315(M⁺+1). Mol. Wt.: 315.

N-[5-(4'-chlorophenyl)-1H-1,2,3-triazol-1-yl]isonicotinamide, (5a), colourless solid, yield 70.01%, mp 202-204°C, R_f 0.73, UV $\lambda_{\max}$ (DMF) 310.6 nm; FTIR (KBr) ν cm⁻¹ 3428 (N-H stretching), 3067 (Ar C-H stretching), 1664 (C=O stretching), 1550 (C=N), 1334 (C-N stretching), 818 (C-Cl stretching); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 5.32 (s, 1H, CH of triazole), 7.64-7.85 (m, 6H, aromatic protons), 8.06 (m, 2H, aromatic protons), 11.83 (s, 1H, CONH). Anal. calcd. for C₁₄H₁₀N₅OCl: C, 60.83; H, 3.53; N, 21.83. Found: C, 60.80; H, 3.54; N, 21.84. FABMS (m/z) 298 (M⁺), 299 (M⁺+1). Mol. Wt.: 299.

N-[5-(4'-dimethylaminophenyl)-1H-1,2,3-triazol-1-yl]isonicotinamide, (5b), colourless solid, yield 77%, mp 196-198°C, R_f 0.77, UV $\lambda_{\max}$ (DMF) 363.2 nm; FTIR (KBr) ν cm⁻¹ 3421 (N-H stretching), 3067 (Ar C-H), 1667 (C=O stretching), 1597 (C=N stretching), 1393 (C-N of triazole); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 3.20 (s, 6H, N(CH₃)₂), 5.58 (s, 1H, C-H of triazole), 7.59-9.28 (m, 8H, aromatic protons), 11.67 (s, 1H, CONH). Anal. calcd. for C₁₆H₁₆N₆O: C, 67.90; H, 5.70; N, 26.24. Found: C, 67.91; H, 5.73; N, 26.39. FABMS (m/z) 307 (M⁺), 308 (M⁺+1). Mol. Wt.: 308.

N-[5-(3',4'-dimethoxyphenyl)-1H-1,2,3-triazol-1-yl]isonicotinamide, (5c), colourless solid, yield 68%, mp 172°C, R_f 0.77, UV $\lambda_{\max}$ (DMF) 363.2 nm; FTIR (KBr) ν cm⁻¹ 3428 (N-H stretching), 3044 (Ar C-H stretching), 2835

(C-O-CH₃ stretching), 1644 (C=O stretching), 1598 (C=N stretching), 1371 (C-N stretching); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 3.93 [s, 6H, (CH₃)₂], 7.10 (s, 1H, C-H of triazole), 7.68-8.77 (m, 7H, aromatic protons), 10.45 (s, 1H, CONH); Anal. calcd. for C₁₆H₁₆N₅O₃: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.79; H, 5.04; N, 19.83. FABMS (m/z) 324(M⁺), 325(M⁺+1). Mol. Wt.: 325.

N-[5-(3'-nitrophenyl)-1H-1,2,3-triazol-1-yl]isonicotinamide, (5d), colourless solid, yield 69%, mp 222-224°C, R_f 0.83, UV $\lambda_{\max}$ (DMF) 292.6 nm; FTIR (KBr) ν cm⁻¹ 3439 (NH stretching of CONH), 3009 (Ar C-H), 1678 (C=O stretching), 1560 (C=N), 1516 (NO₂ stretching); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 5.56 (s, 1H, CH of triazole), 7.59-9.28 (m, 8H, aromatic protons), 12.30 (s, 1H, CONH). Anal. calcd. for C₁₄H₁₀N₆O₃: C, 58.43; H, 3.39; N, 26.21. Found: C, 58.47; H, 3.34; N, 26.25. FABMS (m/z) 309(M⁺), 310(M⁺+1). Mol. Wt.: 310.

N-[5-(2'-nitrophenyl)-1H-1,2,3-triazol-1-yl]isonicotinamide, (5e), colourless solid, yield 81%, mp 218-220°C, R_f 0.77, UV $\lambda_{\max}$ (DMF) 288.7 nm. FTIR (KBr) ν cm⁻¹ 3441 (NH stretching of CONH), 3019 (Ar C-H stretching), 1681 (C=O stretching), 1564 (C=N stretching), 1512 (NO₂ stretching), 1348 (C-N stretching); ¹H NMR (400 MHz DMSO-d₆ δ ppm) 5.85 (s, 1H, CH of triazole), 7.54-7.80 (m, 8H, Ar C-H), 11.02 (s, 1H, CONH). Anal. calcd. for C₁₄H₁₀N₅O₃: C, 58.43; H, 3.39; N, 26.21. Found: C, 58.40; H, 3.35; N, 26.24. FABMS (m/z) 309 (M⁺), 310(M⁺+1). Mol. Wt.: 310.

In vitro antibacterial activity

The primary screen was carried out using the agar disc-diffusion method¹⁶ using Muller-Hinton agar medium. Sterile filter paper discs moistened with the test compound solution in dimethylsulfoxide at concentration of 200 μ g/mL were carefully placed on the agar cultures plates that had been previously inoculated separately with the microorganisms^{17,18}. The plates were incubated at 37°C. The synthesized compounds were tested for their in vitro antimicrobial activity against the Gram-positive bacteria *S. aureus* ATCC 19433 and *B. subtilis* ATCC 6633, the Gram-negative bacteria *E. coli* ATCC 25922, and the filamentous fungus *A. niger* ATCC 1034 and yeast-like pathogenic fungus *C. albicans* ATCC 753. The standards, Ciprofloxacin and Fluconazole were dissolved in dimethylsulfoxide at concentration of 800 μ g/mL. The microorganism suspensions at 10⁶ CFU/mL (colony forming unit/mL) concentrations were inoculated to the corresponding wells. The Petri plates were incubated at 36°C for 24 and 48 h for the bacteria and fungi, respectively. The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganism as detected by unaided eye. The diameters of the growth inhibition zones were measured after 24 h for all microorganisms but after 48 h in case of *C. albicans*.

Preparation of agar wells

A variety of devices such as stainless-steel or ceramic cylinders, single or multiple in design, and paper discs of several sizes are commonly used for antimicrobial assays performed by the agar diffusion method. Holes cut in agar (agar wells) are occasionally useful for the assay of such materials as serum, milk, eggs, or viscous materials. The routine use of agar wells is hampered by the tedium of cutting and removing the agar plugs without disrupting the agar layer¹⁹. Sealing the wells with melted agar is often necessary to obtain uniform inhibition zones²⁰. Agar wells are cut from seeded agar plates by a cylindrical metal tube attached through a trap to a vacuum source. A section of a no. 4 cork borer is a convenient cutter. A most important addition is a 1.5 mm diameter hole in the side of the cutter, about 10 mm from the cutting edge, which acts as a vacuum bypass and prevents tearing the agar from the bottom of the plate. The agar plate is placed in the template on which are marked six evenly spaced locations for the agar wells. The operator cuts the agar with rapid strokes, holding the cutter vertical to assure even contact with the base of the plate. Cutting is greatly simplified if the plates are refrigerated at least 30 min prior to cutting. The firmness of cold agar is very helpful. An agar layer 5 to 6 mm thick makes convenient-size wells.

RESULTS AND DISCUSSION

Chemistry

The synthesis of compounds 3a-f and 5a-e were undertaken as per the scheme 1 and 2. The required 4-amino-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazol (2) was prepared by the action of isonicotinic acid hydrazide and carbodisulfide. The various 4-[[1-(substituted)methylidene]amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazol derivatives (3a-f) were synthesized by condensation of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol with various aromatic aldehydes in dimethyl formamide, in a yield ranging between 31 to 68%. Some N-[5-(4-substituted)-1H-1,2,3-triazol-1-yl]isonicotinamide derivatives (5a-e) were synthesized by a sequence of reactions starting from isonicotinic acid

hydrazide with aromatic aldehydes in glacial acetic acid. ¹H-NMR, Mass and IR spectra were recorded on Bruker DRX-300 (300 MHz), MS Jeol SX-102 (FAB) and BIORAD FTIR Spectrometer instruments respectively. The elemental analysis data of compounds was found to be within ± 0.4 % limit. The IR spectra of final compounds (**3a-f**) and (**5a-e**) showed absorption bands at 2544-2648 cm^{-1} and 1644-1681 cm^{-1} appearing due to the presence of a C=O functional group and a SH functional group respectively, while the band observed at 1516-1598 cm^{-1} corresponds to C=N linkage. Through the ¹H-NMR spectra the synthesis of the compounds (**3a-f**) was confirmed on the basis that -SH proton which was observed as singlet at δ 11.02 - δ 14.02. The CONH proton resonated in compounds (**5a-e**) at δ values between 11.02-14.02 as singlet to broad singlet and this signal was absent when ¹H-NMR was recorded in the presence of D₂O.

Antimicrobial activity

The results revealed that majority of the synthesized compounds showed varying degrees of inhibition against the tested microorganisms. In general, the inhibitory activity against the tested gram-positive bacteria was higher than that of the gram-negative bacteria, and some derivatives showed moderate

or weak activity against *C. albicans* and *A. niger*. The results indicated that the longer the side chain of a compound, the more antimicrobial activity it possesses. Among the benzene ring *ortho*-substitution series, methyl derivatives are more active than the hydroxyl and methoxyl substituted derivatives. However, all the tested compounds exhibited low antifungal activity against *C. albicans*. Moreover, the compounds **3b**, **5a**, **5b** having the side chain showed higher activity than oxime-ether derivatives with the same side chain against *S. aureus*. This bioisosteric replacement resulted in a slightly increased antimicrobial activity. Our study revealed that all the compounds had stronger antibacterial activity against Gram positive bacteria when compared to Gram negative bacteria. The reason for the weaker antifungal activity according to antibacterial effect might be postulated as different action in the mechanism of the compounds such as inhibition effect on respiratory systems of fungal cells, rather than cell wall destruction. The antimicrobial activity revealed that newly synthesized compound **3b**, **5a** and **5b** showed good significant activity. The results of the preliminary antimicrobial testing of the prepared compounds, the typical broad spectrum antibacterial drug Ciprofloxacin (50 $\mu\text{g/mL}$) and the potent antifungal drug Fluconazole (50 $\mu\text{g/mL}$) are shown in Table 1 and 2.

Table 1- Antibacterial activity of compounds.

Compd. No.	Diameter of zone of inhibition (mm)								
	<i>Bacillus subtilis</i>			<i>Escherichia coli</i>			<i>Staphylococcus aureus</i>		
	25 mg ml ⁻¹	50 mg ml ⁻¹	100 mg ml ⁻¹	25 mg ml ⁻¹	50 mg ml ⁻¹	100mg ml ⁻¹	25 mg ml ⁻¹	50 mg ml ⁻¹	100mg ml ⁻¹
2	4.93 ± 0.26	9.86 ± 0.15	15.23 ± 0.15	4.46 ± 0.30	8.8 ± 0.20	14.36 ± 0.15	4.66 ± 0.20	10.76 ± 0.15	16.6 ± 0.20
3a	8.16 ± 0.20	16.13 ± 0.05	20.6 ± 0.20	8.23 ± 0.15	13.16 ± 0.10	19.33 ± 0.20	8.63 ± 0.15	13.83 ± 0.20	20.23 ± 0.20
3b	10.8 ± 0.20	17.86 ± 0.12	22.83 ± 0.15	8.93 ± 0.15	14.80 ± 0.15	20.26 ± 0.15	9.4 ± 0.20	16.4 ± 0.10	21.3 ± 0.10
3c	7.8 ± 0.20	14.96 ± 0.20	19.71 ± 0.20	7.9 ± 0.10	13.83 ± 0.25	18.16 ± 0.20	8.40 ± 0.10	14.63 ± 0.15	19.96 ± 0.15
3d	7.1 ± 0.10	14.13 ± 0.15	19.7 ± 0.10	7.76 ± 0.15	12.8 ± 0.20	18.13 ± 0.05	7.8 ± 0.20	12.63 ± 0.05	19.06 ± 0.25
3e	11.66 ± 0.11	18.26 ± 0.12	23.83 ± 0.15	9.76 ± 0.15	15.63 ± 0.20	20.8 ± 0.10	10.3 ± 0.10	17.36 ± 0.15	22.4 ± 0.20
3f	6.9 ± 0.10	12.93 ± 0.15	18.9 ± 0.10	5.33 ± 0.56	11.45 ± 0.37	16.45 ± 0.00	6.71 ± 0.26	12.56 ± 0.05	18.6 ± 0.20
5a	9.13 ± 0.10	18.86 ± 0.12	22.8 ± 0.20	10.33 ± 0.20	14.7 ± 0.30	20.9 ± 0.20	10.63 ± 0.15	17.4 ± 0.20	23.7 ± 0.10
5b	10.9 ± 0.15	19.7 ± 0.26	23.3 ± 0.17	10.2 ± 0.17	15.16 ± 0.10	21.16 ± 0.10	10.76 ± 0.15	19.6 ± 0.20	24.83 ± 0.15
5c	5.9 ± 0.10	10.76 ± 0.15	16.53 ± 0.12	4.43 ± 0.15	11.36 ± 0.21	17.73 ± 0.25	6.16 ± 0.15	11.63 ± 0.15	17.86 ± 0.11
5d	9.06 ± 0.15	16.23 ± 0.15	21.03 ± 0.15	8.13 ± 0.13	14.13 ± 0.10	20.6 ± 0.20	9.7 ± 0.10	16.7 ± 0.26	22.71 ± 0.26
5e	6.33 ± 0.20	11.03 ± 0.15	18.7 ± 0.10	5.7 ± 0.10	10.7 ± 0.26	15.33 ± 0.20	6.63 ± 0.15	12.70 ± 0.26	18.86 ± 0.12
Ciprofloxacin		26.76 ± 0.15	33.8 ± 0.20		23.32 ± 0.20	29.7 ± 0.26		25.63 ± 0.15	33.43 ± 0.15

Ciprofloxacin was used as standard drug at 50 mg/ml. All the values are in Mean $\pm$ S.D (n=3). Statistical analysis of data was carried out by one-way ANOVA.

Table 2- Antifungal activity of compounds.

Compound No.	Diameter of zone of inhibition (mm)					
	<i>Candida albicans</i>			<i>Aspergillus niger</i>		
	25 mg ml ⁻¹	50 mg ml ⁻¹	100 mg ml ⁻¹	25 mg ml ⁻¹	50 mg ml ⁻¹	100 mg ml ⁻¹
2	7.16 ±0.15	12.5 ±0.10	19.33 ±0.11	6.66±0.11	11.76±0.05	18.16 ±0.20
3a	9.46±0.30	15.7 ±0.26	21.13 ±0.05	8.93±0.05	15.36 ±0.05	20.16 ±0.20
3b	10.5±0.10	16.53±0.31	22.7 ±0.10	10.33±0.41	15.63±0.20	22.16 ±0.20
3c	9.17 ±0.20	14.6 ±0.10	20.7 ±0.26	8.6±0.20	13.2±0.20	20.16 ±0.20
3d	10.3±0.10	13.16 ±0.05	21.7 ±0.26	9.73 ±0.30	16.36±0.20	21.23 ±0.25
3e	11.3±0.10	17.6 ±0.20	24.46 ±0.25	11.03±0.05	17.6 ±0.05	23.8 ±0.20
3f	9.2±0.20	14.23 ±0.25	20.46 ±0.11	8.7±0.26	14.16 ±0.20	20.23 ±0.15
5a	11.13±0.15	18.1 ±0.10	24.16 ±0.15	10.7 ±0.10	17.2 ±0.20	23.73 ±0.05
5b	10.6 ±0.20	18.0 ±0.10	23.1±0.10	10.2 ±0.10	16.56±0.32	22.3 ±0.32
5c	8.63±0.20	15.16±0.20	21.23 ±0.15	8.23±0.15	14.3±0.26	20.5 ±0.10
5d	10.26 ±0.15	17.23±0.15	23.53±0.46	9.6±0.20	16.16±0.20	22.16 ±0.20
5e	9.4 ±0.20	15.43±0.20	22.2±0.10	9.13±0.15	15.63 ±0.20	21.63±0.20
Fluconazole		24.16±0.20	31.56±0.32		23.5±0.10	30.23±0.15

Fluconazole was used as standard at 50 mg/ml. Data are given as Mean ± S.D. (n=3). Statistical analysis of data was carried out by paired t- test.

CONCLUSIONS

Various 4-[{1-(substituted)methylidene}-amino]-3-(4-pyridyl)-5-mercapto-4H-1,2,4-triazol and N-[5-(4-substituted)-1H-1,2,3-triazol-1-yl]isonicotinamide derivatives were synthesized from isonicotinic acid hydrazide. The structure antimicrobial activity relationship of the synthesized compounds, based on the structures of the final compound, which possessed good antimicrobial activity, revealed that the introduction of an aryl moiety generally reduced the antimicrobial activity according to the nature of the aryl moiety. The antimicrobial activities including antibacterial and antifungal properties of the synthesized compounds showed a significant activity as compared with standard drugs.

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