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Synthesis, characterization and *in vitro* biological evaluation of a series of 1,2,4-triazoles derivatives & triazole based schiff bases

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ABSTRACT

The 1,2,4-triazole nucleus is associated with diverse pharmacological activities such as antibacterial, antifungal, hypoglycemic, antihypertensive, analgesic, anti-inflammatory, anti-tumor, anti-viral, urease inhibition and many other properties. The increasing importance of 1,2,4-Triazoles as potent biologically active agents prompted authors to synthesize new series of 4-Amino-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiols. Different 2-(5-Mercapto-3-subsituted-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione(6a-6e) and different Schiff bases 4-[(4-Dimethylamino-benzylidene)-amino]-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol were synthesized from different 4-Amino-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol and characterized by using spectral techniques 1H NMR, 13C NMR, FTIR, and mass spectrometry. All these compounds were screened for antimicrobial activity against Gram Positive, Gram Negative bacteria and fungal stains. Most of these compounds showed good antimicrobial activity. Compound 6a, 6b, 7c, 7a have good activity against P. aeruginosa, E. coli, B. subtilis, & B. cerus. Compound 7d & 7e found to be good active against P. areuginosa. Ciprofloxacin & Fluconazole were used as standard drug.

Key words: 1,2,4- triazole, Schiff bases, Antibacterial activity, Antifungal activity

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INTRODUCTION

The current great interest in preparing functional Antimicrobial materials is inevitably associated with tremendous research efforts dedicated to the design and synthesis of new derivatives of sophisticated heterocyclic compounds. Heterocyclic compounds offer a high degree of structural diversity and have proven to be broadly and economically useful as therapeutic agents [1]. In this context, the Nitrogen containing heterocyclic compounds Azoles such as Isoxazole, Thiazole, Pyrazole, Tetrazole, Triazole are increasingly used in present field of research and development of new compounds. Among these azoles 1,2,4 triazoles are one of the important moiety of medicinal agents which fulfill the requirements of new drug discovery.

The compounds carrying azomethine functional group –C=N– which are known as Schiff bases have gained importance in medicinal and pharmaceutical fields due to the most versatile organic synthetic intermediates and also showing a broad range of biological activities, such as antituberculosis[2], analgesic and anti-inflammatory[3], anticonvulsant[4], antibacterial and antifungal activities[5]. It follows from the literature survey that, depending on the

type of substituent, the derivatives of [1,2,4]triazole have a high potential for biological activity, possessing a wide range of antifungal and antibacterial[6-10], anticonvulsant and antipsychotic[11-13] and antitumour[14-17] properties. The other ones show also anti-inflammatory[18-20], antitubercular[21,22], urease inhibition [23] and antioxidant [24] activities.

As the literature review reviles that 1,2,4 triazole shows a list of biological activity, so this is very important moiety for new drug discovery. There are many ways to synthesize these important class of heterocycles. Khan et al[19] synthesized a series of 4-amino-5-aryl-3H-1,2,4-triazole-3-thiones starting from aromatic acids. In present study we synthesized different 4-amino-5-aryl-3H-1,2,4-triazole-3-thiones and after synthesizing them substituted with pathalic anhydride and 4 amino benzaldehyde to obtain 5 different novel 2-(5-Mercapto-3-subsituted-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione(6a-6e) compounds and 5 different Schiff bases 4-[(4-Dimethylamino-benzylidene)-amino]-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol which was screened for antibacterial and antifungal activity.

MATERIALS AND METHODS

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr pellets) were recorded on a Shimadzu FT-IR 157 spectrophotometer. 1H NMR spectra were recorded on a Perkin–Elmer EM 300 MHz spectrometer using TMS as internal standard. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Reactions were routinely monitored by thin-layer chromatography (TLC) on Silica Gel.

Synthesis of Acid ester (2a-2e). The respective carboxylic acid (0.1mol) was dissolved in methanol (50 mL) in a 100 mL round bottom flask fitted with a reflux condenser. Concentrated sulphuric acid (0.02mol) was added and the reaction mixture subjected to reflux for 8-10 hours. After completion of the reaction the excess methanol was removed and the contents were poured into water, neutralized with sodium carbonate and extracted with diethyl ether (3 × 50 mL). The combined organic layers were dried over anhydrous sodium sulphate and concentrated. (TLC, n-hexane:ethyl acetate; 7 : 3)

Synthesis of acid hydrazide (3a-3e). The aryl ester (0.02 mol) was dissolved in methanol (30 mL), hydrazine hydrate (80 %, 0.04 mol) was added slowly and the reaction mixture was heated under reflux. The reaction was monitored by TLC (petroleum ether : acetone; 8 : 2) and after completion, the reaction mixture was concentrated. The resulting crude solid was filtered, washed with water and recrystallized from aqueous ethanol to give aryl acid hydrazide.

Synthesis of potassium salt of substituted dithiocarbazinic acids (4a-4e). The acid hydrazide (0.01 mol) was added to absolute alcohol (50 mL), containing KOH (1.6 g) at room temperature. Carbon disulphide was added (2.3 g, 0.013mol) and the mixture was stirred at room temperature for 16 h. The mixture was diluted with ether (30 mL) and stirred for further 1hr. The potassium salt was used for the next stage without further purification.

Synthesis of 1,2,4 Triazole (5a-5e). Hydrazine hydrate (99%) (0.02 mol) was gradually added to the above potassium salt (0.01 mol) dissolved in water (20 mL) with stirring and the mixture was refluxed gently for 3 hr, during which hydrogen sulphide evolved and the color of the reaction mixture changed to a dark green color. When evolution of hydrogen sulfide ceased (lead acetate test), It was then cooled to 5 °C and acidified with conc. HCl to pH 1.00. A yellow solid separated out which was filtered, washed with water and was recrystallized from ethanol to make the triazole.

General Synthesis of 2-(5-Mercapto-3-subsituted-1,5-dihydro-[1,2,4]triazole-4-yl)-isoindole-1,3-dione (6a-6e)
A mixture of triazole 5a-5e (0.01 mol) and phthalic anhydride (0.01 mol), in butanol (20 mL) was heated under reflux for 4h. Then the solution was concentrated. Solid product 6a-6e were obtained by filtration which were recrystallized from ethanol.

2-[3-(4-Chloro-phenyl)-5-mercapto-1,5-dihydro-[1,2,4]triazol-4-yl]-isoindole-1,3-dione (6a).
mp 234°C; yield: 58%; IR(KBr, cm^{-1}) : 3124.01s (C-H aromatic), 3000.92s (C-H triazole), 2368.66s (S-H), 1720s (C=O isoindole), 1610.61s (C=N triazole), 1500.67s (N-N), 1346.10s (C-N), 700.0s (C-S), 600.10s (C-Cl); ^{1}H-NMR ($CDCl_3$) δ: 2.2(d, 1H S-H), 5.04 (t, 1H, C-H), 6.80 (s, 1H, N-H), 7.25 (d, 1H), 7.74 (t, 1H), 7.88 (d, 1H), 8.03 (d,1H); ^{13}C-NMR: 50.01 C_5 of Triazole ring, 122.55 C of substituted benzene, 127.18 $C_{2,6}$ of substituted benzene,

128.90 $C_{3,6}$ of benzene, 129.72 $C_{1,2}$ of benzene, 130.03 $C_{4,5}$ of phenyl ring, 138.86 C_3 of triazole ring, 171.75 C=O of Imine; m/z: 359.0(100%), 360.0(43%), 356.0(10%), 339.0(90%).

2-[5-Mercapto-3-(4-nitro-phenyl)-1,5-dihydro-[1,2,4]triazol-4-yl]-isoindole-1,3-dione (6b).
mp 255°C; yield: 59%; IR(KBr, cm^{-1}): 3600.08s, 3066.92s, 2989.76s (C-H), 2929.77s (CH_2), 2789.16s, 2852.81s (N-H), 2368.86s (C-N), 1647.26sc (C=O), 1448.59s (CH_2), 1072.46s (C-N), 1022.31s (C-C), 987.00w (N-H), 848.00s (C-N), 711.76s (C-S); ^{1}H-NMR($CDCl_3$) δ: 2.2(d, 1H S-H), 5.04 (t, 1H, C-H), 6.80 (s, 1H, N-H), 7.60 (d, 1H), 7.82, (t, 1H), 7.86 (d, 1H), 8.03 (d,1H); ^{13}C-NMR: 50.01 C_5 of Triazole ring, 120.25 C of substituted benzene, 127.14 $C_{2,6}$ of substituted benzene, 127.90 $C_{3,6}$ of benzene, 138.44 $C_{1,2}$ of benzene, 146.03 $C_{4,5}$ of phenyl ring, 156.90 C_3 of triazole ring, 172.19 C=O of Imine; m/z: 366.3(50%), 365.3(30%), 338.3(27%).

2-(5-Mercapto-3-pyridin-4-yl-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione (7c).
mp 180°C; yield: 57%; IR(KBr, cm^{-1}) : 3676.45s (C=O), 2900.01s (C-H aromatic), 2850.92s (C-H triazole), 2563.48s (S-H), 1697.41s (C=N), 1610.61s (C=O), 1534.67s (N-N), 1473.66s (C-N), 746.48s (C-S); ^{1}H NMR($CDCl_3$) δ: 2.2(d, 1H S-H), 5.04 (t, 1H, C-H), 6.80 (s, 1H, N-H), 7.88 (d, 1H), 8.03 (d,1H), 8.22 (d, 1H), 8.50 (t, 1H); ^{13}C-NMR: 50.01 C_5 of Triazole ring, 122.55 C of substituted benzene, 127.18 $C_{2,6}$ of substituted benzene, 128.90 $C_{3,6}$ of benzene, 129.72 $C_{1,2}$ of benzene, 130.03 $C_{4,5}$ of phenyl ring, 138.86 C_3 of triazole ring, 171.75 C=O of Imine; m/z: 324.8(100%), 322.2(21%), 323.0(8%).

2-{3-[2-(2,6-Dichloro-phenylamino)-benzyl]-5-mercapto-1,5-dihydro-[1,2,4]triazol-4-yl}-isoindole-1,3-dione (7d).
mp 270°C; yield: 85%; IR(KBr, cm^{-1}) : 3440.04s (N-H triazole), 3198.08s (C-H benzene), 3109.08s (C-H), 2362.88s (S-H), 1590.98s (C=N triazole), 1506.46b (N-H), 1597.11s (N-N), 1300.74s (C-N), 1168.90s (C-C), 840.99s (C-C), 788.91s (C-S), 707.90s (C-Cl); ^{1}H-NMR($CDCl_3$) δ: 2.2(d, 1H S-H), 2.81 (d, 2H, CH_2), 4.18 (s, 1H, N-H), 5.04 (t, 1H, C-H), 6.42 (t, 1H), 6.54 (t, 1H), 6.60 (t, 1H), 6.80 (s, 1H, N-H), 6.94 (t, 1H), 7.23 (d, 1H) 7.88 (d, 1H), 8.03 (d,1H); ^{13}C-NMR: 40.35 C_5 of Triazole ring, 127.75 C_1 of substituted benzene, 128.44 $C_{2,6}$ of substituted benzene, 132.92 $C_{3,6}$ of benzene, 134.34 $C_{1,2}$ of benzene, 159.03 $C_{4,5}$ of phenyl ring, 163.27 C_3 of triazole ring, 165.19 C=O of Imine; m/z: 498.1(100%), 497.1(26%), 484.1(67%),486.3(9%).

[2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid 4-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-5-mercapto-4,5-dihydro-1H-[1,2,4]triazol-3-ylmethyl ester (6e).
mp 260°C; yield: 88%; 3689.95s (C=O), 3300.00s (N-H triazole), 3124.15s (C-H benzene), 2945.40s (C-H cyclic), 2368.66s (S-H), 1830.51s (C=O), 1710.08s (C=N triazole), 1500.46b (N-H), 1437.04s (N-N), 1346.36s (C-N), 1168.90s (C-C), 840.99s (C-C), 792.72s (C-S), 644.25 s (C-Cl); ^{1}H-NMR($CDCl_3$) δ: 2.2(d, 1H S-H), 3.62 (s, 2H, CH_2), 3.94 (s, 2H, CH_2) 4.18 (s, 1H, N-H), 5.04 (t, 1H, C-H), 6.42 (t, 1H), 6.54 (t, 1H), 6.60 (t, 1H), 6.80 (s, 1H, N-H), 6.94 (t, 1H), 7.23 (d, 1H) 7.88 (d, 1H), 8.03 (d,1H); ^{13}C-NMR: 38.68 C_5 of Triazole ring, 124.62 C of substituted benzene, 127.18 $C_{2,6}$ of substituted benzene, 128.90 $C_{3,6}$ of benzene, 129.72 $C_{1,2}$ of benzene, 130.03 $C_{4,5}$ of phenyl ring, 148.86 C_3 of triazole ring, 171.75 C=O of Imine; m/z: 504.0(69%), 505.2(30%), 500.1(9%).

General synthesis of Schiff bases 4-[(4-Dimethylamino-benzylidene)-amino]-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol (7a-7e). A mixture of triazole 5a-5e (0.01 mol) and 4-Dimethylamine benzaldehyde (0.01 mol) in ethanol (25 mL) was treated with concentrated HCl (0.5 mL) and refluxed for 2 h. Once cooled, the reaction mixture was filtered and recrystallized from ethanol to give 7a-7e

5-(4-Chloro-phenyl)-4-[(4-dimethylamino-benzylidene)-amino]-3,4-dihydro-2H-[1,2,4]triazole-3-thiol (7a)
mp 210°C; yield: 87%; IR(KBr, cm^{-1}) : 3649.44s (N-H triazole), 2924.01s (C-H benzene), 2800.88s (C-H), 2332.02s (S-H), 1590.98s (C=N triazole), 1506.46b (N-H), 1390.04s (N-N), 1300.74s (C-N), 1168.90s (C-C), 840.99s (C-C), 783.13s (C-S), 669.32 s (C-Clchlorobenzene); ^{1}H-NMR($CDCl_3$) δ: 2.2(d, 1H S-H), 2.82 (s, 2H, N-H) 5.14 (t, 1H, C-H), 6.46 (d, 1H) 6.80 (s, 1H, N-H), 7.25 (d, 1H), 7.52 (d, 1H), 7.74 (t, 1H), 8.20 (s, 1H, C-H); ^{13}C-NMR: 48.59 CH_3 aliphatic, 48.87 CH_3 aliphatic, 50.01 C_5 of triazole, 108.64 C_1 of substituted benzene, 128.96 $C_{2,6}$ of substituted benzene, 129.74 $C_{3,6}$ of benzene ring, 136.12 $C_{1,2}$ of benzene ring, 151.29 C_4 of benzene ring, 159.57 C_3 of triazole ring, 171.02 C=O of imine; m/z: 360.0(100%), 361.0(43%), 362.0(11%), 330.0(8%).

4-[(4-Dimethylamino-benzylidene)-amino]-5-(4-nitro-phenyl)-3,4-dihydro-2H-[1,2,4]triazole-3-thiol (7b)
mp 180°C; yield: 82%; IR(KBr, cm^{-1}) : 3701.52s (N-H triazole), 2944.01s (C-H benzene), 2800.00s (C-H), 2185.42s (S-H), 1610.00s (C=N triazole), 1532.82b (N-H), 1490.04s (N-N), 1410.04s (C-NO_2 nitrobenzene), 1165.04s (N-N), 815.92r (N-H), 700.18s (C-S); ^{1}H-NMR($CDCl_3$) δ: 2.2(d, 1H S-H), 2.82 (s, 2H, N-H) 5.14 (t, 1H, C-H), 6.46 (d, 1H)

6.80 (s, 1H, N-H), 7.41 (d, 1H), 7.52 (d, 1H), 7.90 (t, 1H), 8.20 (s, 1H, C-H); ^{13}C-NMR: 48.58 CH_3 aliphatic, 49.44 CH_3 aliphatic, 50.00 C_5 of triazole, 108.00 C_1 of substituted benzene, 128.97 $C_{2,6}$ of substituted benzene, 129.74 $C_{3,6}$ of benzene ring, 136.12 $C_{1,2}$ of benzene ring, 151.29 C_4 of benzene ring, 159.57 C_3 of triazole ring, 171.02 C=O of imine; m/z: 369.1(89%), 368.1(28%), 413.0(30%), 364.3(52%).

4-[(4-Dimethylamino-benzylidene)-amino]-5-pyridin-4-yl-3,4-dihydro-2H-[1,2,4]triazole-3-thiol (7c)
mp 160°C; yield: 52%; IR(KBr, cm^{-1}) : 3803.75s (N-H triazole), 3657.16s (C-H benzene), 3050.88s (C-H triazole), 2343.59s (S-H), 1590.98s (C=N triazole), 1521.89s (C-N-C), 1437.04s (N-N), 1375.29s (C-N), 1170.83s (C-C), 1087.99s (C-C), 725.26s (C-S); ^{1}H-NMR($CDCl_3$) δ: 2.2(d, 1H S-H), 2.82 (s, 2H, N-H) 5.14 (t, 1H, C-H), 6.46 (d, 1H) 6.80 (s, 1H, N-H), 7.52 (d, 1H), 8.20 (s, 1H, C-H), 8.26 (d, 1H), 8.50 (t, 1H); ^{13}C-NMR: 48.59 CH_3 aliphatic, 48.87 CH_3 aliphatic, 50.01 C_5 of triazole, 108.64 C_1 of substituted benzene, 128.96 $C_{2,6}$ of substituted benzene, 129.74 $C_{3,6}$ of benzene ring, 136.12 $C_{1,2}$ of benzene ring, 151.29 C_4 of benzene ring, 159.57 C_3 of triazole ring, 171.02 C=O of imine; m/z: 325.4(100%), 323.0(21%), 324.0(10%).

5-[2-(2,6-Dichloro-phenylamino)-benzyl]-4-[(4-dimethylamino-benzylidene)-amino]-3,4-dihydro-2H-[1,2,4]triazole-3-thiol (7d)
mp 230°C; yield: 50%; IR(KBr, cm^{-1}) : 3440.04s (N-H triazole), 3198.08s (C-H benzene), 3109.08s (C-H), 2362.88s (S-H), 1590.98s (C=N triazole), 1506.46b (N-H), 1597.11s (N-N), 1300.74s (C-N), 1168.90s (C-C), 840.99s (C-C), 788.91s (C-S), 707.90s (C-Cl); ^{1}H-NMR($CDCl_3$) δ: 2.2(d, 1H S-H), 2.82 (s, 2H, N-H), 2.92 (d, 2H, CH_2), 4.18 (s, 1H, N-H), 5.14 (t, 1H, C-H), 6.46 (d, 1H) 6.80 (s, 1H, N-H), 7.25 (d, 1H), 7.52 (d, 1H), 7.74 (t, 1H), 8.20 (s, 1H, C-H); ^{13}C-NMR: 48.58 CH_3 aliphatic, 49.44 CH_3 aliphatic, 50.01 C_5 of triazole, 108.64 C_1 of substituted benzene, 127.06 $C_{2,6}$ of substituted benzene, 129.74 $C_{3,6}$ of benzene ring, 136.12 $C_{1,2}$ of benzene ring, 151.29 C_4 of benzene ring, 159.57 C_3 of triazole ring, 171.02 C=O of imine; m/z: 498.4(100%), 490.0(72%), 498.2(24%), 492.1(10%).

[2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid 4-[(4-dimethylamino-benzylidene)-amino]-5-mercapto-4,5-dihydro-1H-[1,2,4]triazol-3-ylmethyl ester (7e)
mp 260°C; yield: 58%; IR(KBr, cm^{-1}) : 3689.95s (C=O), 3300.00s (N-H triazole), 3124.15s (C-H benzene), 2945.40s (C-H cyclic), 2368.66s (S-H), 1830.51s (C=O), 1710.08s (C=N triazole), 1500.46b (N-H), 1437.04s (N-N), 1346.36s (C-N), 1168.90s (C-C), 840.99s (C-C), 792.72s (C-S), 644.25 s (C-Cl); ^{1}H-NMR($CDCl_3$) δ: 2.2(d, 1H S-H), 2.82 (s, 2H, N-H), 2.96 (s, 2H, CH_2), 3.60 (s, 2H, CH_2), 4.18 (s, 1H, N-H), 5.14 (t, 1H, C-H), 6.46 (d, 1H) 6.80 (s, 1H, N-H), 7.25 (d, 1H), 7.52 (d, 1H), 7.74 (t, 1H), 8.20 (s, 1H, C-H); ^{13}C-NMR: 48.59 CH_3 aliphatic, 48.87 CH_3 aliphatic, 50.01 C_5 of triazole, 108.64 C_1 of substituted benzene, 128.96 $C_{2,6}$ of substituted benzene, 129.74 $C_{3,6}$ of benzene ring, 136.12 $C_{1,2}$ of benzene ring, 151.29 C_4 of benzene ring, 159.57 C_3 of triazole ring, 171.02 C=O of imine; m/z: 506.0(100%), 498.4(30%), 498.0(70%), 491.1(10%).

Antibacterial activity
Test Drug Solution: The Test drug solution was prepared by dissolving the compounds in dimethyl sulfoxide (DMSO) with the range of 25, 50, 100 & 200µg/ml.

Preparation of standard solution: Stock solutions of the standard drug (Ciprofloxacin) were prepared in DMSO (Dimethyl sulfoxide) and diluted with DMSO to the concentration of 25µg/ml.

The petridishes were thoroughly washed and sterilized in hot air oven at 160°C for 1hr. 2/3 part of sterile Mueller Hinton Agar media was poured into sterile petridishes for solidifying. Cultured organism (inoculums) were poured in each petridishes. Bores were made on the medium using sterile borer. 0.1mL of test solution (solution of synthesized drug) was added to the respective bores and Cefixime drug used as a standard drug. A control having only DMSO in the cup was maintained in each plate. The petridishes were kept in the refrigerator at 4°C for 15 minutes for diffusion to take place. After diffusion, the petridishes were incubated at 37°C for 24 h and zones of inhibition were observed and measured using a scale.

Antibacterial activity of all the compounds and Standard drug were carried out against all six microorganisms.

Antifungal activity
Test Drug Solution: The Test drug solution was prepared by dissolving the synthesized compounds in dimethyl sulfoxide (DMSO) with the range of 25, 50, 100 & 200µg/ml.

Preparation of standard solution: Stock solutions of the standard drug (Fluconazole) were prepared in DMSO (Dimethyl sulfoxide) and diluted with DMSO to the concentration of 25µg/ml.

The petridishes were thoroughly washed and sterilized in hot air oven at 160°C for 1hr. 30ml of sterile nutrient agar media was poured into sterile petridishes for solidifying. Cultured organism (inoculums) poured in each petridishes. Bores were made on the medium using sterile borer. 0.1ml of test solution (solution of synthesized drug) was added to the respective bores and Fluconazole drug used as a standard drug. A control having only DMF in the cup was maintained in each plate. The petridishes were kept in the refrigerator at 4°C for 15 minutes for diffusion to take place. After diffusion, the petridishes were incubated at 37°C for 24 hours and zones of inhibition were observed and measured using a scale.

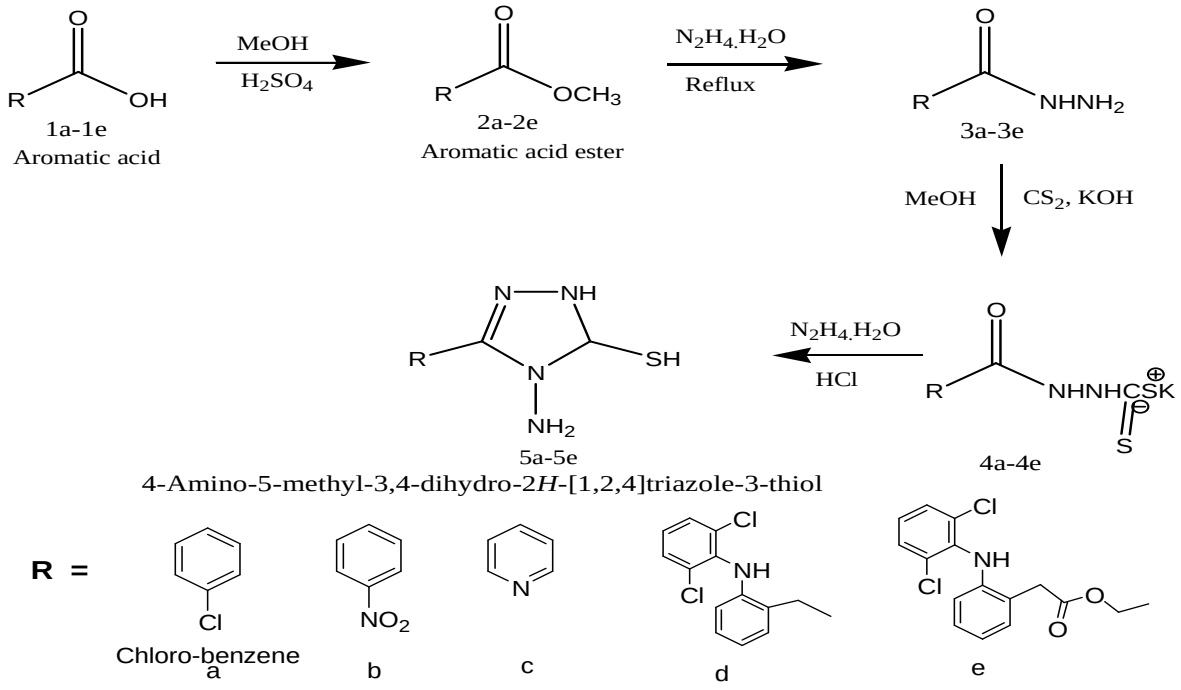
Antifungal activity of all the compounds was carried out against all three microorganisms.

Interpretation: After incubation, the petridishes which showed visible growth were considered to be representing the zone of inhibition in mm. The details of results are furnished in Table 2.

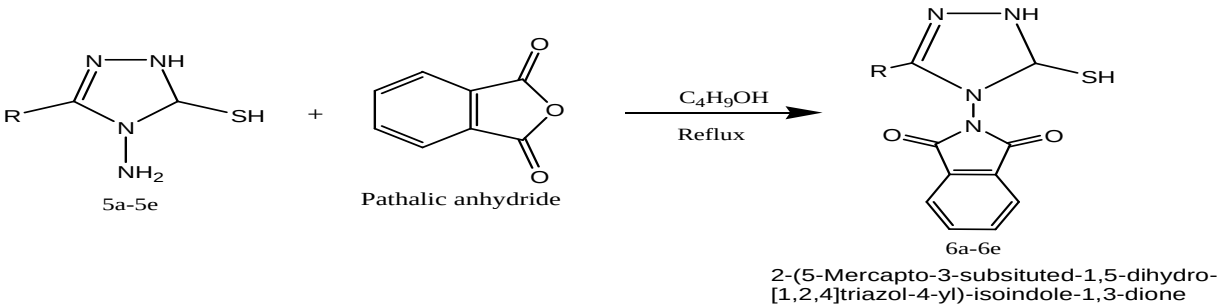
RESULTS AND DISCUSSION

Chemistry

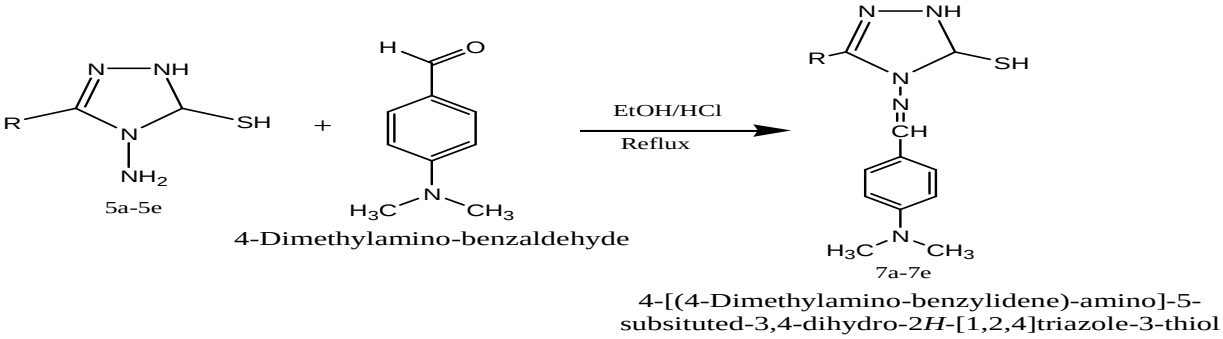
4-Amino-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol(5a-5e) were synthesized by hydrazide of benzoic acid and its different derivatives as per scheme 1 and then these 1,2,4-triazole derivatives reacted with pthalic anhydride (scheme 2) and 4 amino benzaldehyde (scheme 3) to obtain different 2-(5-Mercapto-3-subsituted-1,5-dihydro-[1,2,4]triazol-4-yl)-isoindole-1,3-dione(6a-6e) and different 4-[(4-Dimethylamino-benzylidene)-amino]-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol (7a-7e) respectively. Newly synthesized compounds were characterized by IR, NMR, Mass spectral analyses. The physicochemical properties of synthesized compound are given in Table 1.



Scheme 1: Synthesis of different 4-Amino-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol



Scheme 2: Synthesis of 2-(5-Mercapto-3-subsituted-1,5-dihydro-[1,2,4]triazole-4-yl)-isoindole-1,3-dione



Scheme 3: Synthesis of different Schiff bases 4-[(4-Dimethylamino-benzylidene)-amino]-5-subsituted-3,4-dihydro-2H-[1,2,4]triazole-3-thiol

Table 1: Physicochemical properties of synthesized compounds

COMPOUND CODE	MOLECULAR FORMULA	MOLICULAR WEIGHT	MELTING POINT ·C	ClogP
6a	$C_{16}H_{11}ClN_4O_2S$	358.80	234	3.5917
6b	$C_{16}H_{11}N_5O_4S$	369.35	255	2.6216
6c	$C_{15}H_{11}N_5O_2S$	325.35	180	1.3816
6d	$C_{23}H_{17}Cl_2N_5O_2S$	498.38	270	6.5865
6e	$C_{25}H_{19}Cl_2N_5O_4S$	556.42	260	6.6078
7a	$C_{17}H_{18}ClN_5S$	359.88	210	4.808
7b	$C_{17}H_{18}N_6O_2S$	370.43	180	3.838
7c	$C_{16}H_{18}N_6S$	326.42	160	2.598
7d	$C_{24}H_{24}Cl_2N_6S$	499.49	230	7.74
7e	$C_{26}H_{26}Cl_2N_6O_2S$	557.49	260	7.423

In the IR spectra, the appearance of a comparatively weak band in the region 1,605–1,506 cm-1 for C=N, at the expense of strong absorption for the carbonyl of hydrazides, indicated the formation of triazoles. Furthermore, the appearance of a C=S absorption band in the region 1,352–1,309 cm-1 indicated that the triazoles are in their thione form. In 1H NMR spectra, a broad downfield singlet in the region d = 7.00-7.80 ppm was assigned to the NH proton. A signal in the region 4.81–5.80 ppm, integrating to two protons, was assigned to NH_2 protons. In 13C NMR spectra, the signals in the regions d = 177.6–166.2 and 161.9–147.1 ppm were assigned to C-3 and C-5 of the triazole nucleus, respectively. Four or six signals for aromatic carbons were observed due to substitution on the benzene ring. The structures were further confirmed by mass-spectral analysis. The molecular ion peak was observed for all the compounds.

Antimicrobial activity All the synthesized compounds were tested for their in-vitro growth inhibitory activity against a panel of standard strains of pathogenic microorganism including Gram positive bacteria (*Staphylococcus aures*, *Bacillus subtilis* and *Bacillus cerus)*, Gram negative bacteria (*Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*) and fungal strains (*Aspergillus*, *A. fumigates* and *C. albicans*). Stock solutions of the synthesized compounds were prepared in DMSO and then from this stock solution 4 concentration of 25 µg/ml, 50 µg/ml, 100 µg/ml and 200 µg/ml prepared by dilution Stock solutions of standard drug (Ciprofloxacin & Fluconazole) were also prepared in DMSO and 100 µg/ml concentration used for antimicrobial activity.

Compounds also show good activity against *A. fumigtus & S. aures.* Antibacterial and antifungal activity of all compounds at 100 µg/ml given in the Table 2 and Table 3 respectively. The antimicrobial activity data shows that compound 6a have better antibacterial and antifungal activity. Compound 6a, 6b, 7c, 7a have good activity against *P. aeruginosa, E. coli, B. subtilis*, & *B. cerus*. Compound 7d & 7e found to be good active against *P. areuginosa*. Compound 7c shows excellent activity against *A. Fumigatus* than standard drug fluconazole.

Table 2: Antibacterial activity of synthesized compounds

Compound	Zone of inhibition in mm					
	Antibacterial activity at 100 µg/ml					
	B. s	B. c	S. a	E. c	P. v	P. a
6a	14 ± 0.30	16 ± 0.16	11 ± 0.46	12 ± 1.05	13 ± 0.74	17 ± 0.59
6b	14 ± 0.65	15 ± 0.34	12 ± 1.19	12 ± 0.56	12 ± 1.04	14 ± 0.57
6c	13 ± 0.25	14 ± 0.15	11 ± 0.54	09 ± 0.78	14 ± 0.97	15 ± 0.28
6d	13 ± 0.42	11 ± 0.63	10 ± 0.51	12 ± 0.35	10 ± 0.28	13 ± 0.65
6e	10 ± 1.03	14 ± 0.26	11 ± 0.32	12 ± 0.91	13 ± 0.36	16 ± 0.78
7a	15 ± 0.60	11 ± 0.85	13 ± 0.29	10 ± 0.26	09 ± 0.68	13 ± 1.17
7b	12 ± 0.37	11 ± 0.22	12 ± 1.02	12 ± 1.26	11 ± 0.56	14 ± 0.73
7c	12 ± 0.43	13 ± 1.04	11 ± 0.24	10 ± 0.58	11 ± 0. 82	14 ± 0.47
7d	08 ± 0.71	10 ± 0.36	07 ± 0.65	09 ± 0.39	10 ± 0.27	13 ± 0.68
7e	10 ± 0.27	09 ±0.76	12 ± 0.23	09 ± 0.87	11 ± 0.32	12 ± 0.34
Control DMSO	--	--	--	--	--	--
Ciprofloxacin	18 ± 0.33	18 ± 0.42	19 ± 0.53	21 ± 0.37	20 ±0.26	15 ± 0.56

Table 3: Antifungal activity of synthesized compounds

Compound	Zone of inhibition in mm		
	Antifungal activity at 100 µg/ml		
	C. albicans	Aspergillus	A. fumigatus
6a	19 ± 0.66	22 ± 0.47	18 ± 0.59
6b	19 ± 0.54	19 ± 0.39	18 ± 0.66
6c	17 ± 0.37	15 ± 0.57	17 ± 0.49
6d	18 ± 0.75	17 ± 0.63	16 ± 0.32
6e	17 ± 0.68	15 ± 0.79	16 ± 0.27
7a	17 ± 0.26	16 ± 0.28	18 ± 0.91
7b	16 ± 0.86	17 ± 1.06	17 ± 0.28
7c	20 ± 0.92	22 ± 0.93	26 ± 0.27
7d	20 ± 0.26	11 ± 0.39	12 ± 1.14
7e	18 ± 0.36	13 ± 0.46	15 ± 0.23
Control DMSO	--	--	--
Fluconazole	24 ± 0.37	22 ± 0.26	20 ± 034

CONCLUSION

Ten different derivatives of 1,2,4 triazole was synthesized as per the scheme and their structure was confirmed by FT IR, NMR , Mass analysis. All the synthesized compounds shows good antibacterial and antifungal activity at 100 µg/ml. compound 6a, 6b, 7c, 7a have good activity against *P. aeruginosa, E. coli, B. subtilis*, & *B. cerus*. Compound 7d & 7e found to be good active against *P. areuginosa*. On the other hand all the synthesized compounds show very good activity against fungal strains specially *Aspergillus* and *C. albicans*.

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