



Green Synthesis and Evaluation of 5-(4-Aminophenyl)-4-Aryl-4H-1, 2, 4-Triazole-3-Thiol Derivatives

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Abstract

The green synthesis of 5-(4-aminophenyl)-4-aryl-4H-1,2,4-triazole-3-thiol was achieved in four steps. In the first step, 4-amino benzoic acid was refluxed in ethanol along with catalyst Conc. Sulphuric acid to produce ethyl-4-amino benzoate **I**. Further compound **I** was refluxed with hydrazine hydrate in ethanol to produce 4-amino benzohydrazide **II**. Compound **II** was refluxed in ethanolic potassium hydroxide with carbon disulfide to produce 5-(4-aminophenyl)-1, 3, 4-oxadiazole-2-thiol **III**. Compound **III** refluxed in ethanol with different substituted primary aryl amine gave title compounds 5-(4-aminophenyl)-4-aryl-4H-1, 2, 4-triazole-3-thiol **IVa-o**. The compounds obtained were identified by FT-IR, ¹H-NMR, GC- mass spectroscopy data, and elemental analysis and also screened for *in-vivo* antimicrobial activity. *In vitro* antibacterial activity was carried out against organisms like *E.coli*, *K.pneumonia*, *S.aureus*, and *B.subtilis* as well as *in vitro* antifungal activity were carried out against organisms like *A.niger* and *S.cerevisiae*. *In vitro* antimicrobial evaluation, the most potent broad spectrum compound IVc, IVd, and IVf were found significant agent against standard drug Norfloxacin and Ketoconazole.

Keywords: Triazole, Antimicrobial, MIC, FT-IR, ¹H-NMR, GC-MS.

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infections in human beings, the mortality and morbidity caused by microbes have an alarming worldwide impact on the human population due to the increasing number of multidrug-resistant and the upsurge of multi-drug resistant had made the treatment of microbial infections complicated [1-5].

Considering the requirement of today's scenario of synthetic chemistry, there is a need of new synthetic methods which should prepare a diverse range of molecules in limited time. The microwave assisted technique is one

1. Introduction

In spite the existence of a number of drugs that are used for the treatment of bacterial

of the methods which can fulfill this requirement [6]. Nowadays, many organic syntheses can be performed with the help of microwave technique. It has many advantages over the traditional methods viz. Enhanced reaction rate, elevation in amount of end product and reduction in the time period required for completion of the reaction [7]. Microwave technique also has a greener approach in organic synthesis. The reduction in chemical waste and reaction times in organic synthesis and chemical transformations are achievable due to the use of MW techniques in combination with greener reaction media [8].

Green synthesis achieved by microwave assisted organic synthesis and microwave decline generation of hazardous products, reduces energy consumption, utilization of less solvents, and atom economy [9].

Heterocycles containing an azole ring system are found to exhibit a wide spectrum of biological activities, including antibacterial and antifungal properties. Triazole nucleus is an important pharmacophore appear extensively in various types of pharmaceutical agents and bioactive molecules, such as anti-HIV [10], antitubercular [11], anti-inflammatory, anticancer [12], antimicrobial [13-15], anticonvulsant [16], antimalarial [17], analgesic [18], antiviral [19], antidiabetic [20], and anti-trypanosomal [21].

1, 2, 4-Triazoles are among the most promising heterocyclic compounds play an important role in medicinal and agricultural filed. The substituted 1, 2, 4-triazole

derivatives have been reported to possess antimicrobial activity mainly due to N-C-S linkage in the skeleton [22] and several drugs containing 1, 2, 4-triazole group i.e. Fluconazole, Flupoxam, and Anastrozole etc are well known [23]. Substituted-1, 2, 4-triazoles, is among the various heterocyclic compounds that have received considerable attention during the last two decades for their potential broad spectrum of pharmacological activities which include antifungal (23), antioxidant [24], antibacterial (25-30), anticancer (31), anti-inflammatory and analgesic (32), prompted by these observations, it was contemplated to synthesize some new 5-(4-aminophenyl)-4-aryl-4*H*-1,2,4-triazole-3-thiol derivatives with a view to explore their potency as better chemotherapeutic agents. The structures of all the synthesized compounds were confirmed by FT-IR, ¹H-NMR, GC-mass spectroscopy and elemental analysis.

2. Materials and Methods

2.1. Materials

All reagents and solvents used in the present study were of analytical grade and purchased from Loba chemie (India).The progress of the reactions were monitored by TLC using Merck silica gel precoated plates, with appropriate mobile phase, visualization by iodine vapor and UV chamber and product were purified by recrystallization technique. All the melting points were recorded on a Veego apparatus (Mumbai, India) and were

uncorrected. All the green synthesized compounds were characterized by their FT-IR, $^1\text{H-NMR}$, GC Mass spectroscopy. FT-IR spectra were recorded in KBr on Bruker FT-IR instrument (Germany), $^1\text{H-NMR}$ spectra were recorded on Bruker Avance $^1\text{H-NMR}$ spectrometer (Germany), at 400 MHz in DMSO-d₆, by using varian instrument using TMS as internal standard and chemical shift values are given in ppm downfield to TMS (tetramethylsilane) and GC Mass were recorded on GCMS-QP-5050 Shimadzu (Japan). The standard drugs Norfloxacin, and Ketoconazole were obtained as gift samples from Macleod's Pharma. Ltd., Daman, India.

2.2. Synthesis of Ethyl-4-Amino Benzoate (I)

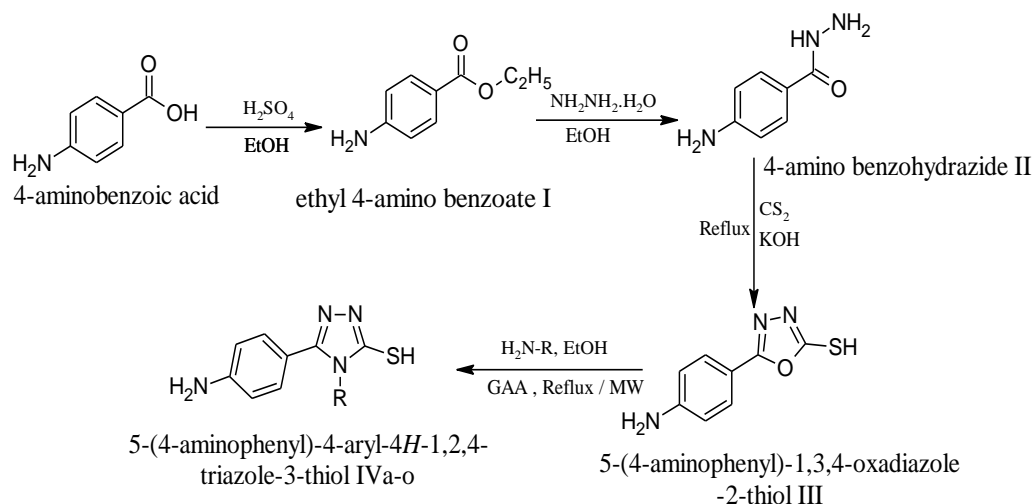
In a 50 mL round bottom flask, 4-amino benzoic acid (0.01 mol, 1.37 g) was dissolved into 20 mL dry ethanol, while stirring; 0.5 mL of conc. H_2SO_4 was added dropwise to obtain precipitate and further reaction mixture was refluxed on water bath for 01 h, cooled to RT. Cooled reaction solution was neutralized with 10 % Na_2CO_3 until the pH is approximately 08 and extracted twice with 10 mL portions of dichloromethane. Dichloromethane layer was washed twice with 08 mL water; dichloromethane solution dried over anhydrous sodium sulfate and organic solvent was removed using the rotary evaporator, dried, recrystallized using a solvent pair, ethyl alcohol and water (1:1) and air dried. M.P: 88-89 °C, Yield 64.10 % (Scheme 1) [33].

2.3. Synthesis of 4-amino Benzohydrazide (II)

In a 50 mL round bottom flask, ethyl 4-amino benzoate I (0.01 mol, 1.65g) and hydrazine hydrate 99 % (0.01mol, 03 mL) were dissolved into 20 mL ethanol; further refluxed on water bath for 01 h and allowed to stand overnight. The precipitated obtained, filtered, washed with water, dried and recrystallized using methanol. M.P: 226-227 °C, Mobile phase- Ethyl acetate: Diethyl Ether: Water (3.1: 1.2: 5.7), Yield 65.40 % (Scheme 1) [33].

2.4. Synthesis of 5-(4-aminophenyl)-1, 3, 4-oxidazole-2-thiol (III)

In a 50 mL round bottom flask, 4-amino benzohydrazide II (0.01 mol, 1.51 g) was dissolved into a solution of potassium hydroxide (0.012 mol) in 10 mL ethanol; to this solution carbon disulfide (0.02 mol, 06 mL) was added with shaking, and the reaction mixture was refluxed on water bath for 15 h. The solvent was removed under reduced pressure; precipitate then poured into cold water; acidified with dil. HCl, filtered, washed with water, dried and recrystallized using ethanol. M.P: 234 °C, Yield 55.00 % (Scheme 1) [34].



Scheme 1. Synthesis of 5-(4-aminophenyl)-4-aryl-4H-1, 2, 4-triazole-3-thiol.

2.5. Synthesis of 5-(4-Aminophenyl)-4-Aryl-4H-1, 2, 4-Triazole-3-Thiol (IV) (Method of Synthesis-Conventional / MW* Assisted)

In a 50 mL round bottom flask, 5-(4-aminophenyl)-1, 3, 4-oxidazole-2-thiol III (0.01 mol, 1.93 g), primary aromatic amine (0.01 mol) and three drops of glacial acetic acid were dissolved into 50 mL absolute ethanol and solution was refluxed for 15 h or subjected to microwave at 4 level (280W), then reaction mixture was poured into ice cold water, the precipitate filtered, washed with water, dried and recrystallized using ethanol [35].

2.5.1. 5-(4-Aminophenyl)-4-(1, 3-Benzothiazol-2-Yl)-4H-1, 2, 4-Triazole-3-Thiol (Iva)

Yield %: 55.10 (16 h), 80.22 (12 min)*, M.P.: 184-186 °C, TLC: Toluene: Methanol (7:3), R_f: 0.71. FT-IR ν max (KBr, cm⁻¹): 1376 (C-N str, ring), 1502 (C=N str, aromatic), 2916 (S-H str), 1601, 3436 (N-H str, amine); ¹H NMR (DMSO, 400 MHz) δ : 5.82 (s, H, NH₂), 6.64-7.90 (m, 4H, benzthiazole) 7.50-8.56 (m, 4H, aromatic), 14.11 (s, 1H, SH). MS: [M]⁺ at m/z 325; Analytical Calculations for Formula; C₁₅H₁₁N₅S₂, Predicted: C, 55.36; H, 3.41; N, 21.52, Found: C, 55.42; H, 3.39; N, 21.58.

2.5.2. 5-(4-aminophenyl)-4-(5-nitro-1, 3-benzothiazol-2-yl)-4H-1, 2, 4-triazole-3-thiol (IVb)

Yield %: 48.35 (16 h), 77.42 (14 min)*, M.P.: 202-204 °C, TLC: Toluene: Methanol (7:3), *R_f*: 0.63. FT-IR ν max (KBr, cm^{-1}): 1345, 1645(C-NO₂ str), 1512 (C=N str, aromatic), 1645 (C-N str, ring), 2847 (S-H str), 1604, 3434 (N-H str, amine); ¹H NMR (DMSO, 400 MHz) δ : 5.15 (s, H, NH₂), 6.58-7.94 (m, 3H, benzthiazole) 7.40-8.88 (m, 4H, aromatic), 13.44 (s, 1H, SH); MS: [M]⁺ at m/z 370; Analytical Calculations for Formula; C₁₅H₁₀N₆S₂O₂, Predicted: C, 48.64; H, 2.72; N, 22.69, Found: C, 48.60; H, 2.76; N, 22.65.

2.5.3. 5-(4-aminophenyl)-4-(4-chloro-6-nitro-1, 3-benzothiazol-2-yl)-4H-1, 2, 4-triazole-3-thiol (IVc)

Yield %: 51.32 (18 h), 79.65 (16 min)*, M.P.: 170-172 °C, TLC: Toluene: Methanol (7:3), *R_f*: 0.69. IR ν max (KBr, cm^{-1}): 703(C-Cl str), 1337 (C-N str, ring), 1499 (C=N str, aromatic), 1345(C-NO₂ str), 2833 (S-H str), 1604, 3446 (N-H str, amine); ¹H NMR (DMSO, 400 MHz) δ : 5.67 (s, H, NH₂), 6.46-8.08 (m, 4H, aromatic), 8.58-8.90 (m, 2H, benzthiazole), 14.21 (s, 1H, SH); MS: [M]⁺, [M+2] at m/z 404, 406; Analytical Calculations for Formula; C₁₅H₉ClN₆O₂S₂, Predicted: C, 44.50; H, 2.24; N, 20.76, Found: C, 44.53; H, 2.20; N, 20.71.

2.5.4. 5-(4-aminophenyl)-4-(6-chloro-1, 3-benzothiazol-2-yl)-4H-1, 2, 4-triazole-3-thiol (IVd)

Yield %: 57.22 (18 h), 86.71 (16 min)*, M.P.: 160-161 °C, TLC: Toluene: Methanol (7:3), *R_f*: 0.67. FT-IR ν max (KBr, cm^{-1}): 729 (C-Cl str), 1366 (C-N str, ring), 1497 (C=N str, aromatic), 2746 (S-H str), 1602, 3437 (N-H str, amine); ¹H NMR (DMSO, 400 MHz) δ : 5.81(s, H, NH₂), 6.62-8.22 (m, 4H, aromatic), 8.05-8.30 (m, 3H, benzthiazole), 13.70 (s, 1H, SH); MS: [M]⁺, [M+2] at m/z 359, 361; Analytical Calculations for Formula; C₁₅H₁₀ClN₅S₂, Predicted: C, 50.06; H, 2.80; N, 19.46, Found: C, 50.10; H, 2.77; N, 19.48.

2.5.5. 5-(4-aminophenyl)-4-(4, 6-dinitro-1, 3-benzothiazol-2-yl)-4H-1, 2, 4-triazole-3-thiol (IVe)

Yield %: 46.48 (15 h), 77.16 (12 min)*, M.P.: 190 °C, TLC: Toluene: Methanol (7:3), *R_f*: 0.52. FT-IR ν max (KBr, cm^{-1}): 1345 (C-N str, ring), 1517 (C=N str, aromatic), 1337, 1603(C-NO₂ str), 2915 (S-H str), 1603, 3336 (N-H str, amine); ¹H NMR (DMSO, 400 MHz) δ : 5.50 (s, H, NH₂), 6.44-8.08 (m, 4H, aromatic), 9.35-9.50 (m, 2H, benzthiazole), 14.32 (s, 1H, SH); MS: [M]⁺ at m/z 415; Analytical Calculations for Formula; C₁₅H₉N₇O₄S₂, Predicted: C, 43.37; H, 2.18; N, 23.60, Found: C, 43.40; H, 2.20; N, 23.61.

2.5.6. 5-(4-Aminophenyl)-4-(4-Nitro-1, 3-Benzothiazol-2-Yl)-4H-1, 2, 4-Triazole-3-Thiol (Ivf)

Yield %: 56.79 (17 h), 79.11 (15 min), M.P.: 195 °C, TLC: Toluene: Methanol (7:3), R_f: 0.76. FT-IR v max (KBr, cm⁻¹): 1329 (C-N str, ring), 1506 (C=N str, aromatic), 1329, 1604(C-NO₂ str), 2744(S-H str), 1604, 3344 (N-H str, amine); ¹H NMR (DMSO, 400 MHz) δ: 5.48 (s, H, NH₂), 6.50-8.12 (m,4H, aromatic), 7.85-8.60 (m,3H, benzthiazole), 14.11 (s,1H, SH); MS: [M]⁺ at m/z 370; Analytical Calculations for Formula; C₁₅H₁₀N₆S₂O₂, Predicted: C, 48.64; H,2.72; N,22.69 , Found: C, 48.72; H, 2.66; N, 22.73.

2.5.7. 5-(4-Aminophenyl)-4-(6-Nitro-1, 3-Benzothiazol-2-Yl)-4H-1, 2, 4-Triazole-3-Thiol (Ivg)

Yield %: 51.36 (15 h), 90.21 (12 min)*, M.P.: 234-235°C, TLC: Toluene: Methanol (7:3), R_f: 0.45. FT-IR v max (KBr, cm⁻¹): 1352 (C-N str, ring), 1500 (C=N str, aromatic), 1352 (C-NO₂ str), 2913(S-H str), 1603, 3347 (N-H str, amine); ¹H NMR (DMSO, 400 MHz) δ: 5.56 (s, H, NH₂), 6.46-8.20 (m,4H, aromatic), 7.73-8.67(m,3H, benzthiazole), 14.23 (s,1H, SH); MS: [M]⁺ at m/z 370; Analytical Calculations for Formula; C₁₅H₁₁N₆S₂O₂, Predicted: C, 48.64; H,2.72; N,22.69 , Found: C, 48.70; H,2.68; N,22.75.

2.5.8. 2-[3-(4-Aminophenyl)-5-Sulfanyl-4H-1, 2, 4-Triazole-4-Yl]-1, 3-Benzothiazole-6-Carboxylic Acid (Ivh)

Yield %: 59.33 (16 h), 87.06 (14 min)*, M.P.: 265-267 °C, TLC: Toluene: Methanol (7:3), R_f: 0.49. FT-IR v max (KBr, cm⁻¹): 1328 (C-N str, ring), 1516 (C=N str, aromatic), 1605 (C=O str), 2949 (S-H str), 1605, 3344 (N-H str, amine); ¹H NMR (DMSO, 400 MHz) δ: 5.82 (s, H, NH₂), 6.38-8.18 (m,4H, aromatic), 7.69-8.58 (m,3H, benzthiazole), 11.12 (s,1H, COOH), 14.13 (s,1H, SH); MS: [M]⁺ at m/z 369; Analytical Calculations for Formula; C₁₆H₁₁N₅S₂O₂, Predicted: C, 52.02; H,3.00; N,18.96, Found: C, 52.06; H, 3.04; N, 18.95.

2.5.9. 3-[3-(4-Aminophenyl)-5-Sulfanyl-4H-1, 2, 4-Triazole-4-Yl] Phenol (Ivi)

Yield %: 60.23 (16 h), 88.43 (14 min)*, M.P.: 230-231°C, TLC: Toluene: Methanol (7:3), R_f: 0.55. FT-IR v max (KBr, cm⁻¹): 1346 (C-N str, ring), 1520 (C=N str, aromatic), 2746 (S-H str), 1603, 3349 (N-H str, amine), 3437 (C-OH str) ; ¹H NMR (DMSO, 400 MHz) δ: 14.29 (s,1H, SH), 5.55 (s,1H,OH), 5.72 (s, H, NH₂), 6.60-8.85 (m,8H, aromatic); MS: [M]⁺ at m/z 284; Analytical Calculations for Formula; C₁₄H₁₂N₄OS, Predicted: C, 59.14; H,4.25; N,19.70, Found: C, 59.18; H, 4.27; N, 19.77.

2.5.10. 4-[3-(4-Aminophenyl)-5-Sulfanyl-4H-1, 2, 4-Triazole-4-Yl] Phenol (Ivj)

Yield %: 67.26 (17 h), 78.51 (15 min)*, M.P.: 201-202 °C, TLC: Toluene: Methanol (7:3), *R_F*: 0.42. FT-IR ν max (KBr, cm^{-1}): 1345 (C-N str, ring), 1511 (C=N str, aromatic), 2740(S-H str), 1604, 3334 (N-H str, amine), 3450 (C-OH str); ^1H NMR (DMSO, 400 MHz) δ : 5.41 (s,1H,OH), 5.67 (s, H, NH_2), 6.50-8.81 (m,8H, aromatic), 14.18 (s,1H, SH); MS: $[\text{M}]^+$ at *m/z* 284; Analytical Calculations for Formula; $\text{C}_{14}\text{H}_{12}\text{N}_4\text{SO}$, Predicted: C, 59.14; H,4.25; N,19.70 , Found: C, 59.16; H,4.24; N,19.68.

2.5.11. 5-(4-Aminophenyl)-4-(Pyridine-2-Yl)-4H-1, 2, 4-Triazole-3-Thiol (Ivk)

Yield %: 57.39 (15 h), 66.58 (12 min)*, M.P.: 208-210°C, TLC: Toluene: Methanol (7:3), *R_f* : 0.60. FT-IR ν max (KBr, cm^{-1}): 1351 (C-N str, ring), 1513 (C=N str, aromatic), 2833(S-H str), 1604, 3344 (N-H str, amine); ^1H NMR (DMSO, 400 MHz) δ : 5.47 (s, H, NH_2), 6.54-7.92 (m, 4H, aromatic), 7.90-8.57(m, 4H, pyridine), 14.26 (s,1H, SH); MS: $[\text{M}]^+$ at *m/z* 269; Analytical Calculations for Formula; $\text{C}_{13}\text{H}_{11}\text{N}_5\text{S}$, Predicted: C, 57.97; H,4.12; N,26.00, Found: C, 57.92; H, 4.17; N, 26.03.

2.5.12. 5(4-Aminophenyl)-4-(6-Bromo-1, 3-Benzothiazol-2-Yl)-4H-1, 2, 4-Triazole-3-Thiol (Ivl)

Yield %: 65.46 (15 h), 71.08 (12 min)*, M.P.: 196-197 °C, TLC: Toluene: Methanol (7:3), *R_F*: 0.33. FT-IR ν max (KBr, cm^{-1}): 812 (C-Br str), 1345 (C-N str, ring), 1511 (C=N str, aromatic), 2833 (S-H str), 1604, 3347 (N-H str, amine); ^1H NMR (DMSO, 400 MHz) δ : 5.73(s, H, NH_2), 6.48-8.04 (m,4H, aromatic), 7.71-8.42 (m,3H, benzthiazole), 14.05 (s,1H, SH); MS: $[\text{M}]^+$, $[\text{M}+2]$ at *m/z* 404, 406; Analytical Calculations for Formula; $\text{C}_{15}\text{H}_{10}\text{BrN}_5\text{S}_2$, Predicted: C, 44.56; H,2.49; N,17.32, Found: C, 44.57; H, 2.47; N, 17.34.

2.5.13. 2-[3-(4-Aminophenyl)-5-Sulfanyl-4H-1, 2, 4-Triazole-4-Yl] Phenol (Ivm)

Yield %: 58.31 (16 h), 70 (14 min)*, M.P.: 204 °C, TLC: Toluene: Methanol (7:3), *R_F*: 0.84. FT-IR ν max (KBr, cm^{-1}): 1350 (C-N str, ring), 1516 (C=N str, aromatic), 2760(S-H str), 1604, 3350 (N-H str, amine), 3425 (C-OH str); ^1H NMR (DMSO, 400 MHz) δ : 5.39 (s,1H,OH), 5.62 (s, H, NH_2), 6.90-8.65 (m, 8H, aromatic), 14.31 (s,1H, SH) ; MS: $[\text{M}]^+$ at *m/z* 284; Analytical Calculations for Formula; $\text{C}_{14}\text{H}_{12}\text{N}_4\text{SO}$, Predicted: C, 59.14; H,4.25; N,19.70 , Found: 59.10; H,4.25; N,19.73.

2.5.14. 1-{3-[3-(4-Aminophenyl)-5-Sulfanyl-4H-1, 2, 4-Triazole-4-Yl] Phenyl} Ethanone (Ivn)

Yield %: 55.16 (17 h), 62.44 (15 min), M.P.: 206 °C, TLC: Toluene: Methanol (7:3), *RF*: 0.37. FT-IR ν max (KBr, cm^{-1}): 1327 (C-N str, ring), 1518 (C=N str, aromatic), 1620 (C=O str), 2961(S-H str), 1605, 3358 (N-H str, amine); ^1H NMR (DMSO, 400 MHz) δ : 2.55 (s, 3H, CH_3), 5.49 (s, H, NH_2), 7.18-8.22 (m, 8H, aromatic), 14.46 (s, 1H, SH); MS: $[\text{M}]^+$ at m/z 310; Analytical Calculations for Formula; $\text{C}_{16}\text{H}_{14}\text{N}_4\text{OS}$, Predicted: C, 61.92; H, 4.55; N, 18.05, Found: C, 61.96; H, 4.57; N, 18.08.

2.5.15. 1-{2-[3-(4-Aminophenyl)-5-Sulfanyl-4H-1, 2, 4-Triazole-4-Yl] Phenyl} Ethanone (Ivo)

Yield %: 68.29 (17 h), 73.14 (15 min)*, M.P.: 180-184 °C, TLC: Toluene: Methanol (7:3), *RF*: 0.29. FT-IR ν max (KBr, cm^{-1}): 1333 (C-N str, ring), 1528 (C=N str, aromatic), 1629 (C=O str), 2944 (S-H str), 1601, 3536 (N-H str, amine); ^1H NMR (DMSO, 400 MHz) δ : 2.44 (s, 3H, CH_3), 5.40 (s, H, NH_2), 7.63-8.12 (m, 3H, benzthiazole) 6.53-8.07 (m, 4H, aromatic), 14.33 (s, 1H, SH); MS: $[\text{M}]^+$ at m/z 367; Analytical Calculations for Formula; $\text{C}_{17}\text{H}_{13}\text{N}_5\text{OS}_2$, Predicted: C, 55.57; H, 3.57;

N, 19.06, Found: C, 55.60; H, 3.59; N, 19.08.

2.6. Biological Evaluation

2.6.1. Antimicrobial Evaluation

All the synthesized compounds (**IVa-o**) were screened for their *in vitro* antimicrobial activity against two gram positive species *S.aureus* (*S.aureus*, NCIM 2079), *B.subtillis* (*B.subtillis*, NCIM 2711) and two gram negative strains *E.coli* (*E.coli*, NCIM 2685), *K.pneumonia* (*K.pneumoniae*, NCIM 2957) and two fungal species *A.nigar* (*A.nigar*, NCIM 596), *S.cerevisiae* (*S.cerevisiae*, NCIM 3102) for antifungal evaluation, using the broth dilution method. Minimum inhibitory concentration (MIC) was determined through serial dilution of stock solution and visually observed, interpretation of growth and compared with standard reference Norfloxacin for antibacterial and Ketoconazole for antifungal evaluation (Table 1) [1, 3].

3. Results and Discussion

The scheme of synthesis for 5-(4-aminophenyl)-4-aryl-4H-1, 2, 4-triazole-3-thiol derivatives are depicted in Scheme 1 and were produced by conventional and micro wave assisted method. Application of green chemistry i.e. micro wave assisted method found to be useful because atom

economy, conservation of energy and solvents, purity of product, and speed up reaction time. The FT-IR spectra for compounds **IVa-o** were recorded and reveals that functional groups in the target compounds are appeared at their characteristic frequency C-Cl str, between 703-729 cm^{-1} , C-Br str, at 812 cm^{-1} , C-N, str. ring 1327-1646 cm^{-1} , C=N str, aromatic 1497-1528 cm^{-1} , C=O str, 1605-1629 cm^{-1} , SH str, 2740-2961 cm^{-1} , C-NO₂ str, 1345-1604 cm^{-1} , N-H, str. 3334-3536 cm^{-1} , C-OH str, 3425-3450 cm^{-1} , etc. The chemical shift (δ) for δ value for methyl, three hydrogen was observed in the range of 2.44-2.55 ppm, hydroxyl hydrogen was observed in the range of 5.39-5.41 ppm, δ value for primary amino two hydrogen's were observed in the range of 5.15-5.82 ppm, δ value for aromatic hydrogen's were observed in the range of 6.38-8.88 ppm, δ value for benzthiazole hydrogen's were observed in the range of 6.58-9.90 ppm, δ value for pyridine hydrogen's were observed in the range of 7.90-8.07 ppm, δ value for carboxyl hydrogen was observed at 11.12 ppm, δ value for mercapto hydrogen was observed in the range of 13.44-14.46 ppm. The m/e value was observed, e.g., in case of IVa-o at 269-415 (M)⁺. So, from the physical and spectral data, we could conclude that the desired compounds synthesized successfully.

3.1. Antimicrobial Evaluation

The results are alluding to Table 1 and indicate the following:

1. *In vitro* antibacterial evaluation of newly synthesized target compound **IVc** found most potent and broad spectrum compound at MIC of 26, 38 and 24, against *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus subtilis* respectively. Compound substituted with 6-chloro-1, 3-benzothiazol-2-yl, on triazole ring (**Vd**) was found significant agent in newly synthesized series.
2. *In vitro* antifungal evaluation of newly synthesized target compound **IVc** found most significant and broad spectrum agent at MIC of 28, and 22 against *Aspergillus niger*, and *Saccharomyces cerevisiae* respectively. Compound substituted with 4-nitro-1,3-benzothiazol-2-yl on triazole ring (**IVf**) was found significant agent in newly synthesized series.

Thus, from the obtained antibacterial and antifungal activity data we could conclude that 5-(4-aminophenyl)-4-aryl-4H-1,2,4-triazole-3-thiol are good antimicrobial agents when they are substituted with 1, 3-benzothiazol-2-yl along with the electron-withdrawing groups i.e., (-Cl, and -NO₂) substituted at specific position are contributing positively for activity.

Table 1. Minimum inhibitory concentration values ($\mu\text{g/mL}$) of 5-(4-aminophenyl)-4-aryl-4H-1, 2, 4-triazole-3-thiol compounds (IVa-o) against microbes.

IV

Comp	R	<i>E. coli.</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>A. niger</i>	<i>S. cerevisiae</i>
IVa		37	59	55	42	49	47
IVb		43	63	76	55	63	53
IVc		26	38	64	24	28	22
IVd		53	49	62	38	51	56
IVe		40	66	75	53	47	34
IVf		31	70	63	49	38	27
IVg		52	43	80	67	73	35
IVh		50	77	68	61	87	44
IVi		39	50	71	79	55	28
IVj		64	72	93	80	63	
IVk		36	48	82	84	66	39
IVl		57	69	33	45	70	33
IVm		47	80	58	66	97	42
IVn		58	76	91	39	71	69
IVo		61	88	97	76	98	59
IV	---	38	45	72	35	39	30
Norfloracin	---	16	30	25	15	---	---
Ketoconazole	---	---	---	---	---	22	14

4. Conclusion

We have synthesized a series of 1, 2, 4-triazole-3-thiol analogues conjugated with different substituted 1, 3-benzothiazol-2-yl, pyridine, and phenols by conventional and microwave assisted method. The synthesized compounds were evaluated for their characterization, antimicrobial activity by spectroscopy and minimum inhibitory concentration method. Coupling of different substituted 1, 3-benzothiazol-2-yl, pyridine, and phenol enhance the antimicrobial activity. Among the analogues 1, 2, 4-triazoles conjugated with substituted 1, 3-benzothiazol-2-yl, pyridine, and phenol substituted with electron-withdrawing groups exhibited more potent antimicrobial agents.

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