Original Research Article

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES OF CERTAIN 1,2,4-TRIAZOLE DERIVATIVES

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ABSTRACT

A new class of 1,2,4-triazole derivatives namely 3-(3,4-substituted-phenyl)-4-(4-fluoro-phenyl)-5-methyl-4H-1,2,4-triazoles (6a-6h) were synthesized. The structure of each novel compound was elucidated on the basis of elemental analysis, IR and ^{1}H NMR spectral data. The pharmacological properties of 1,2,4-triazoles were enhanced by introducing alkyl, alkoxy and halogen substituents. The minimum inhibitory concentration was evaluated by broth dilution method. As predicted and as evidenced in the literature, the halogen substituted compounds were found to be better antimicrobial agents.

Keywords: 1,2,4-Triazoles, Structural characterization, Anti bacterial activity, Anti fungal activity, Broth dilution method.

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INTRODUCTION

1,2,4-triazole is a basic aromatic heterocycle that has a five-membered ring containing two carbon atoms and three nitrogen atoms. 1,2,4-triazoles have drawn the attention of medicinal chemists due to its low toxicity and good pharmacokinetic and pharmaco dynamic profiles. Their stability to metabolic degradation, affinity for various bio targets, increased solubility in metabolic systems [1] impart them the wide pharmacological spectrum [2-11]. 1,2,4-triazole constitute the promising therapeutic agents and is the core structural component [12] of many drugs. Added to its significant pharmacological features, the incorporation of various aromatic and aliphatic substituents, especially those containing halogen atoms enhance the antimicrobial activity. Prompted by these observations, the authors synthesized 1,2,4-triazole derivatives attached to various alkyl groups, halogens etc. and evaluated for their antimicrobial activity.

MATERIALS AND METHODS

All Chemicals and regents were procured from Ranbaxy Laboratories Ltd, Chemical Division, India. The standard bacterial and fungal strains were procured from National Centre for Cell Sciences, Pune, India. Nutrient broth, nutrient agar and 5 mm diameter antibiotic assay discs were obtained from Hi-Media Laboratories Limited, India. Synthesized

compounds were recrystallized using suitable solvent. Melting points were determined by Scientific melting point apparatus, India and uncorrected. UV-Visible spectrophotometer manufactured by Shimadzu Corporation, Japan was used for transparency measurements. Infrared spectra of the compounds were recorded on Perkin-Elmer FT-IR spectrometer (υ_{max} in cm^{-1}). ^1H NMR spectra were recorded on a JOEL (300MHz) spectrometer using TMS as an internal standard (chemical shifts in δ). Mass spectra were recorded on a mass spectrometer JOEL sx-102.

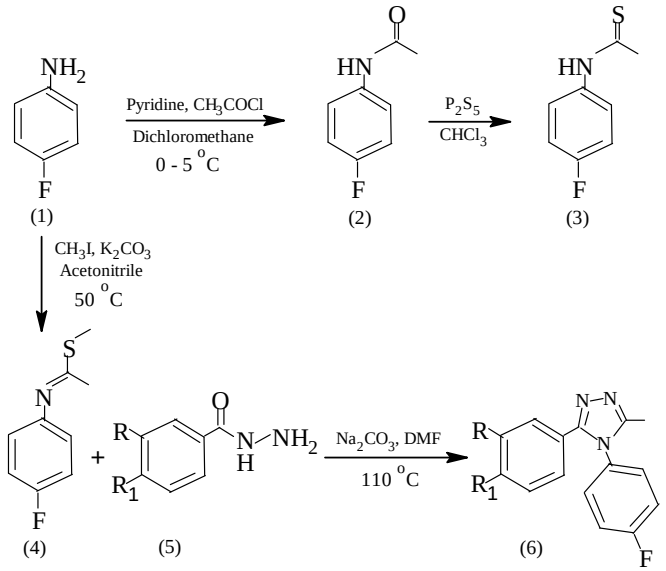
General synthetic procedures

Synthesis of N-(4-fluorophenyl)-acetamide (2)

Pyridine (20.6 mL) followed by acetyl chloride (7.4 mL) was added to a solution of 4–fluoro aniline (1) (10 g) in dichloromethane (200 mL) at 0 °C. The reaction mixture was stirred at 0 °C – 5 °C for 30 min. The progress of the reaction was monitored by TLC. The reaction mixture was diluted with water and extracted with dichloro methane. The organic layer was washed with 10% hydrochloric acid, 10% sodium bicarbonate solution, water, brine solution, dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to get crude N-(4-fluorophenyl) acetamide (2).

Synthesis of N–(4–fluoro-phenyl)-thioacetamide (3)

Phosphorus pentasulphide (18.12 g) was added to the solution of 2 (11.5 g) in chloroform (250 mL) and the reaction mixture was stirred for 5 hours. The progress of the reaction was monitored by TLC. The reaction mixture was diluted with chloroform and filtered. Chloroform layer was concentrated under reduced pressure to get crude N–(4–fluoro-phenyl)-thioacetamide (3).



Compound	6a	6b	6c	6d	6e	6f	6g	6h	6i	6j
R_1	Br	Cl	CH_3	OCH_3	F	Cl	F	CH_3	OCH_3	H
R	H	H	H	H	H	Cl	F	CH_3	OCH_3	NO_2

Scheme 1: Synthesis of 3-(3,4-substituted-phenyl)-4-(4-fluoro-phenyl)-5-methyl-4H-1,2,4-triazoles (6a-6j)

Synthesis of N-(4-fluoro-phenyl) –thioacetamidic acid methyl ester (4)

Potassium carbonate (18.5 g) followed by methyl iodide (8.7 mL) was added to a solution of 3 (8.3 g) in acetonitrile (200 mL). The reaction mixture was stirred at 50 °C for 2 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure to get the crude form. The crude compound was diluted with ethyl acetate, ethyl acetate layer was washed with water, brine solution and dried over anhydrous sodium sulphate. The excess solvent was removed under reduced pressure to get 4.

Synthesis of 3-(3-bromo-phenyl)-4-(4-fluoro-phenyl)-5-methyl-4H-1,2,4-triazole (6a)

To the solution of 4 (6 g) in dimethyl formamide (100 mL), 4-bromo-benzoice acid hydrazide (4.2 g) and sodium carbonate (100 mg) was added and heated to 110 °C for 24 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature, poured into ice cold water and extracted with ethyl acetate. The ethyl acetate layer was washed with water, brine solution, dried over anhydrous sodium sulphate and the solvent was removed under reduced pressure to get crude 3-(3-bromo-phenyl)-4-(4-fluoro-phenyl)-5-methyl-4H-1,2,4-triazole (6a). Similar procedure was extended for the synthesis of 6b – 6j. The reaction sequence leading to the formation these compounds (6a – 6j) is out lined in Scheme 1.

Broth Dilution Method [13]

Minimum Inhibitory Concentration (MIC) was found out by broth dilution method. Standardized inoculum (matched to McFarland $BaSO_4$ standard) of suspension of organisms were prepared. A series of glass tubes containing different concentrations of 1,2,4-triazoles dissolved in DMSO and spiller in nutrient broth were incubated with one drop of inoculum and mixed gently by shaking the rack. Two growth control tubes were also prepared without the addition of test compound and its optical density was determined. The tubes were incubated for 24 hours at 37 ^oC in air. The turbidity produced in each tube was recorded by UV-Visible spectrophotometer. The turbidity produced by the broth (without inoculum) was considered to be 100% transparency. The minimum inhibitory concentration was noted as the concentration of the 1,2,4-triazole, that completely inhibits the growth of the microorganism i.e. 100% transparency.

RESULTS AND DISCUSSION

The 1,2,4-triazol derivatives synthesised were characterised by elemental analysis, IR ^1H NMR, mass spectral and elemental analysis.

Characterisation of N-(4-fluorophenyl)-acetamide (2)

Molecular formula, yield, Element Calculated% (Found%): C_8H_8FNO, 76%, C 62.74 (61.98); H 5.26 (5.49); N 9.15 (9.67); O 10.45 (10.68), IR ν_{max} in cm^{-1} (Group): 3050 (aromatic -CH_2-); 2980 (aliphatic -CH_2-); 1654 (>C=O), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.18 (s, 3H, -CH_3), 7.15 (broad peak, 1H, >NH), 7.27-7.29 (d, 2H, Ar-H), 7.44-7.46 (d, 2H, Ar-H).

Characterisation of N–(4–fluoro-phenyl)-thioacetamide (3)

Molecular formula, yield, Element Calculated% (Found%): C_8H_8FNS, 79%, C 56.78 (57.12); H 4.77 (4.31); N 8.28 (8.83); S 18.95 (18.56), IR ν_{max} in cm^{-1} (Group): 3225 (>NH); 3050 (aromatic -CH_2-); 2980 (aliphatic -CH_2-); 1134 (>C=S), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.18 (s, 3H, CH_3), 7.15 (broad s, 1H, >NH), 7.27-7.29 (d, 2H, Ar-H), 7.44-7.46 (d, 2H, Ar-H).

Characterisation of N-(4-fluoro-phenyl) –thioacetamidic acid methyl ester (4)

Molecular formula, yield, Element Calculated% (Found%): $C_9H_{10}FNS$, 62%, C 58.99 (58.02); H 5.50 (5.45); N 7.64 (8.19); S 17.50 (18.20), IR ν_{max} in cm^{-1} (Group): 3050 (aromatic $-CH_2-$); 2980 (aliphatic $-CH_2-$); 1650 (>C=N), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 7.11-7.13 (d, 2H, Ar-H); 6.62-6.64 (d, 2H, Ar-H); 2.31 (s, 3H, S-CH_3); 1.97 (s, 3H, CH_3).

Characterisation of 3-(3,4-substituted-phenyl)-4-(4-fluoro-phenyl)-5-methyl-4H-1,2,4-triazoles (6a-h)

6a: R_1=Br, R=H, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{15}H_{11}BrFN_3$, 46, 130-133, 54.24(54.86); 3.34(3.05); 5.72(5.12); 12.65(12.94), IR ν_{max} in cm^{-1} (Group): 1608(>C=N-); 1625(>N-N<); 2936(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.34 (s, 3H, CH_3); 7.24 (d, 2H, Ar-H), 7.60 (d, 2H, Ar-H) ; 7.46 (s, 1H, Ar-H); 7.40-8.22 (m, 3H, Ar-H).

6b: R_1=Cl R=H, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{15}H_{11}ClFN_3$, 50, 123-125, 62.62(62.91); 3.85(3.29); 6.60(6.90); 14.60(14.27), IR ν_{max} in cm^{-1} (Group): 1610(>C=N-); 1626(>N-N<); 2939(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.35 (s, 3H, CH_3); 7.24 (d, 2H, Ar-H), 7.60 (d, 2H, Ar-H); 8.01 (s, 1H, Ar-H); 7.40-8.16 (m, 3H, Ar-H).

6c: R_1=Me, R=H, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{16}H_{14}FN_3$, 51, 128-130, 71.89(71.28); 5.28(5.81); 7.11(7.29); 15.72(15.27), IR ν_{max} in cm^{-1} (Group): 1605(>C=N-); 1624(>N-N<); 2935(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.37 (s, 3H, CH_3); 3.10 (s, 3H, CH_3); 7.24 (d, 2H, Ar-H); 7.60 (d, 2H, Ar-H); 7.79 (s, 1H, Ar-H), 7.19–8.09 (m, 3H, Ar-H).

6d: R_1=OMe, R=H, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{16}H_{14}FN_3O$, 50, 144-146, 67.83(67.12); 4.98(5.64); 6.71(6.90); 14.83(14.37), IR ν_{max} in cm^{-1} (Group): 1610(>C=N-); 1625(>N-N<); 2937(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.44 (s, 3H, CH_3); 3.83 (s, 3H, CH_3); 7.60 (d, 2H, Ar-H); 7.24 (d, 2H, Ar-H); 7.36 (s, 1H, Ar-H); 6.95–7.84 (m, 3H, Ar-H).

6e: R_1=F, R=H, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{15}H_{11}F_2N_3$, 57, 127-129, 66.42(62.16); 4.09(4.90); 14.01(14.92); 15.49(14.93), IR ν_{max} in cm^{-1} (Group): 1608(>C=N-); 1623(>N-N<); 2941(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.44 (s, 3H, CH_3); 7.60 (d, 2H, Ar-H); 7.24 (d, 2H, Ar-H); 7.52 (s, 1H, Ar-H); 7.20–8.05 (m, 3H, Ar-H).

6f: R_1=Cl, R=Cl, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{15}H_{10}C_{l2}FN_3$, 50, 119-121, 55.92(55.20); 3.13(3.08); 5.90(6.57); 13.04(12.91), IR ν_{max} in cm^{-1} (Group): 1612(>C=N-); 1620(>N-N<); 2944(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.44 (s, 3H, CH_3); 7.60 (d, 2H, Ar-H); 7.24 (d, 2H, Ar-H); 7.95 (s, 1H, Ar-H); 7.49–8.00 (m, 2H, Ar-H).

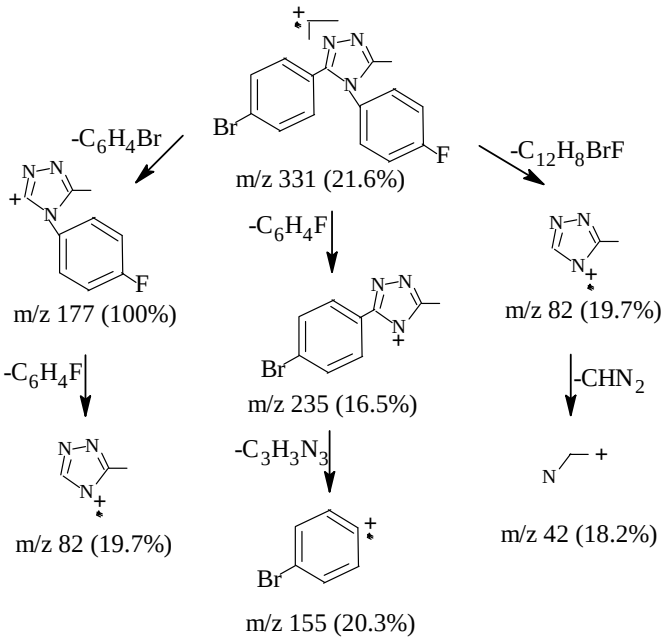
6g: R_1=F, R=F, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{15}H_{10}F_3N_3$, 45, 133-135, 62.28(62.58); 3.48(3.87); 19.70(20.48); 14.53(14.57), IR ν_{max} in cm^{-1} (Group): 1605(>C=N-); 1627(>N-N<); 2943(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.44 (s, 3H, CH_3); 7.60 (d, 2H, Ar-H); 7.24 (d, 2H, Ar-H); 7.50 (s, 1H, Ar-H); 7.28–7.54 (m, 2H, Ar-H).

6h: R_1=Me, R=Me, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{17}H_{16}FN_3$, 50, 144-145, 72.58(72.50); 5.73(5.49); 6.75(6.88); 14.94(14.57), IR

ν_{max} in cm^{-1} (Group): 1608(>C=N-); 1626(>N-N<); 2945(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.44 (s, 3H, CH_3); 7.60 (d, 2H, Ar-H); 7.24 (d, 2H, Ar-H); 2.34 (6H, s, Ar–CH_3); 7.67 (s, 1H, Ar-H); 7.17–8.33 (m, 2H, Ar-H).

6i: R_1=OMe, R=OMe, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{17}H_{16}FN_4O_2$, 50, 119-120, 65.17(65.27); 5.15(5.27); 6.06(6.27); 13.41(13.57), IR ν_{max} in cm^{-1} (Group): 1610(>C=N-); 1624(>N-N<); 2950(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.44 (s, 3H, CH_3); 7.60 (d, 2H, Ar-H); 7.24 (d, 2H, Ar-H); 3.83 (6H, s, Ar–OCH_3); 7.25 (s, 1H, Ar-H); 6.94–7.53 (m, 2H, Ar-H).

6j: R=H, R_1=NO_2, Molecular Formula, Yield(%), m.p(^{0}c), Element Calculated(%) Found(%): $C_{15}H_{11}F\ N_4O_2$, 45, 133-134, 60.40(60.47); 3.72(3.57); 6.37(6.19); 18.78(18.92), IR ν_{max} in cm^{-1} (Group): 1608(>C=N); 1626(>N-N<); 2948(>CH_2), ^{1}HNMR (300 MHz, DMSO-d_6) δ ppm: 2.44 (s, 3H, CH_3); 7.60 (d, 2H, Ar-H); 7.24 (d, 2H, Ar-H); 8.05 (2H, d, Ar-H); 8.32 (d, 2H, Ar-H).



Scheme 2: The mass spectra of 3-(3-bromo-phenyl)-4-(4-fluoro-phenyl)-5-methyl-4H-1,2,4-triazole (6a)

Mass Spectra

The mass spectrum of 3-(3-bromo-phenyl)-4-(4-fluoro-phenyl)-5-methyl-4H-1,2,4-triazole (6a) exhibits the molecular ion ($M^{+\cdot}$) peak at m/z 331(21.6%). Mass spectral fragmentation of 6a is presented in Scheme 2. The base peak was observed at m/z 177(100%), other prominent peaks were appeared at m/z 42(18.2%), 82(19.7%), 155(20.3%), 235(16.5%).

Antimicrobial activity

The antibacterial studies were carried out against selected bacteria and fungi. The gram positive bacteria screened were *Staphylococcus aureus* NCCS 2079 and *Bacillus cereus* NCCS 2106. The gram negative bacterial screened were *Escherichia coli* NCCS 265 and *Pseudomonas aeruginosa* NCCS2200. The fungi screened were *Aspergillus niger* nccs 1196 and *Candida albicans* NCCS 3471. The minimum inhibitory concentration was found by broth dilution method. The results shown in the Table 1 reveal that all 1,2,4-triazoles under study except methyl derivatives were active against the tested microbes. The antibacterial

activity was compared with that of standards namely, cefaclor where as the antifungal activity with ketoconazole. However none of them demonstrated superior activity to that of standards. It was interesting to note that among all the 1,2,4-triazole derivatives under study, those containing halogen (6a, 6b, 6e, 6f and 6g), -NO_2 (6j), and –OCH_3 (6d and 6i) group were found to exhibit significant antimicrobial activity. 1,2,4-triazole derivatives containing -CH_3 substituent (6c and 6h) was found to exhibit trivial antibacterial activity but their antifungal activity was comparable to the compounds under study.

Table 1: Antimicrobial activity of 3-(3,4-substituted-phenyl)-4-(4-fluoro-phenyl)-5-methyl-4H-1,2,4-triazoles (6a-6j)

Compound	Minimum inhibitory concentration (µg/mL)					
	***Staphylococus aureus* NCCS 2079**	***Bacillus Cereus* NCCS 2106**	***Escherichia coli* NCCS 2065**	***Pseudomanas aeruginos* NCCS 2200**	***Aspergillus niger* NCCS 1196**	***Candida albicans* NCCS 2106**
6a	21.56	18.34	22.18	22.49	15.26	16.34
6b	16.48	18.24	17.38	17.24	14.62	14.82
6c	>50	>50	>50	>50	38.24	38.40
6d	28.62	32.18	30.26	34.22	22.49	23.76
6e	15.22	15.76	15.22	16.42	12.54	12.62
6f	12.26	15.42	12.26	12.98	10.24	11.58
6g	11.28	10.24	11.34	11.98	9.27	8.42
6h	>40	>40	>40	>40	31.54	36.52
6i	24.58	18.52	27.18	26.54	16.24	16.56
6j	16.38	18.28	18.62	17.20	13.54	14.06
Cefaclor	2	4	3	3	------	------
Ketoconazole	-----	-----	-----	-----	0.75	0.40

CONCLUSION

Nine novel 1,2,4-triazoles were synthesized employing the simple synthetic route, their structures have been confirmed by elemental analysis, IR and ^{1}H NMR spectral data. Antibacterial investigation was carried out against bacteria namely, *S. aureus, P.aeruginosa, E.coli and B.subtili,* and the antifungal investigation carried out against fungi namely *C.albicans and A.nigerz.* Minimum inhibitory concentration was found out by broth dilution method. Antibacterial and antifungal studies revealed that compounds containing halogen, -NO_2, and –OCH_3 groups were potent antimicrobial agents than other tested compounds.

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