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Synthesis and antimicrobial activity evaluation of some substituted *N*'[arylidene]-2-(3-methyl-5-pyridin-4-yl-1*H*-1,2,4-triazol-1-yl) acetohydrazide compounds

Shantaraman G. Khanage[1]*, Popat B. Mohite[1], Ramadas B. Pandhare[1], Appala S. Raju[2]

ABSTRACT

A new class of Schiff bases of 4-(3-methyl-1H-1,2,4-triazol-5-yl) pyridine was synthesized to meet structural requirements essential for the antimicrobial activity. 4-(3-methyl-1H-1,2,4-triazol-5-yl)pyridine was reacted with ethyl chloroacetate to form ethyl [3-methyl-5-(pyridin-4-yl)-1H-1,2,4-triazol-1-yl] acetate (2). Compound 2 was treated with hydrazine hydrate in methanol to yield 2-[3-methyl-5-(pyridin-4-yl)-1H-1,2,4-triazol-1-yl] acetohydrazide (3). The condensation of compound 3 with various aromatic aldehydes yielded the corresponding substituted *N*'-[arylidene]-2-(3-methyl-5-pyridin-4-yl-1H-1,2,4-triazol-1-yl) acetohydrazide (4a-j). The structural assessment of the newly synthesized Schiff bases (4a-j) was carried out by FT-IR, ^{1}H-NMR, Mass spectra and elemental analysis. The target compounds 4a-j were screened for their *in vitro* antimicrobial activity and the Minimum Inhibitory Concentration of each compound was determined by liquid broth method. From newly synthesized series compounds 4b, 4c and 4h were exhibited significant antimicrobial potential against *Staphylococcus aureus* NCIM 2079, *Escherichia coli* NCIM 2065, *Candida albicans* NCIM 3471 and *Aspergillus niger* NCIM 1196.

Keywords: *Schiff bases, antimicrobial, aromatic aldehydes, elemental analysis.*

1. INTRODUCTION

1,2,4-triazoles has received substantial attention owing to their effective biological importance like anticancer [1], antibacterial [2,3], anticonvulsant [4], anti-inflammatory, analgesic [5], antifungal [6,7], antidepressant [8], antituberculine [9], antimalarial [10] and hypoglycemic [11] activities. Compounds containing azomethine group is known as Schiff bases. Day by day Schiff bases are more frequently applied for the better treatment of human welfare. Schiff bases are important class of compounds due to their flexibility, structural similarities with natural biological substances and also due to presence of imine (-N=CH-) which imports in elucidating the mechanism of transformation and rasemination reaction in biological system. Literature survey shows that numerous Schiff bases exhibits biological activities such as antimicrobial, antifungal [12,13], antihypertensive [14], antianxiety, antidepressant [15], anticancer [16], anti-inflammatory [17] activities. In continuation of our research on synthesis and pharmacological assessment of 3,5-disubstituted 1,2,4-triazole [18-23] and appraisal of the above mentioned facts we decided to

[1] Department of Pharmaceutical chemistry, M.E.S. College of Pharmacy, Sonai, Tq-Newasa, Dist.-Ahmednagar, Maharashtra, India-414105

*Corresponding author e-mail address: ***shantaram1982@gmail.com***

[2] Department of Pharmaceutical chemistry, H. K. E.'S College of Pharmacy, Sedam road, Gulbarga, Karnataka, India-585105.

synthesize and evaluate *in vitro* antimicrobial activity of some novel Schiff bases of *N*'-[arylidene]-2-(3-methyl-5-pyridin-4-yl-1H-1,2,4-triazol-1-yl)acetohydrazide (4a-j).

2. EXPERIMENTAL SECTION

2.1 Synthesis protocol

2.1.1 Synthesis of 4-(3-methyl-1H-1,2,4-triazol-5-yl)pyridine (1). Isonicotinic acid hydrazide (0.14 mole) was dissolved in methanol, to this solution acetamide (0.14 mole) was added and resulting reaction mixture refluxed on water bath for 3 hrs, then cooled to room temperature to get crude residue of compound 1, the obtained product was recrystallized from ethanol with an yield 89%.

2.1.2 Synthesis of ethyl [3-methyl-5-(pyridin-4-yl)-1H-1,2,4-triazol-1-yl]acetate (2). An equimolar mixture of 4-(3-methyl-1H-1,2,4-triazol-5-yl)pyridine (compound 1, 0.09 mole), ethyl chloroacetate (0.09 mole) and anhydrous potassium carbonate (0.09 mole) in methanol (40 ml) was refluxed on a water bath for 4 hrs, cooled to room temperature, filtered, dried and recrystallized from ethanol. The compound was separated as white residue with an yield 82%.

2.1.3 Synthesis of 2-[3-methyl-5-(pyridin-4-yl)-1H-1,2,4-triazol-1-yl]acetohydrazide (3). To a mixture of compound 2 (0.05 mole) in methanol (40 ml), hydrazine hydrate (99%, 0.05 mole) was added with continuous stirring to get clear solution. Then resulting reaction mixture was refluxed on water bath for about 5 hrs. The solution was concentrated and allowed to cool overnight. The resulting solid obtained was filtered, washed with cold ethanol, dried and recrystallized from ethanol. The compound was separated as white residue with an yield 73%.

2.1.4 General procedure for the synthesis of N'-[arylidene]-2-(3-methyl-5-pyridin-4-yl-1H-1,2,4-triazol-1-yl)acetohydrazide (4a-j). Equimolar quantity of the acetohydrazide (compound 3, 0.008 mole) and various aromatic aldehydes (0.008 mole) were taken, to this reaction mixture, 1-2 drops of hydrochloric acid and ethanol:dioxane mixture (50 ml) were added then reaction mixture was heated on water bath for consecutive 8 hrs. Then reaction mixture poured in to crush ice to get corresponding schiff bases (4a-j). The precipitate thus obtained was filtered washed with cold water and purified by recrystallization from ethanol. The physical and spectral data of the compounds (4a-j) are described in Tables 1 and 2 respectively.

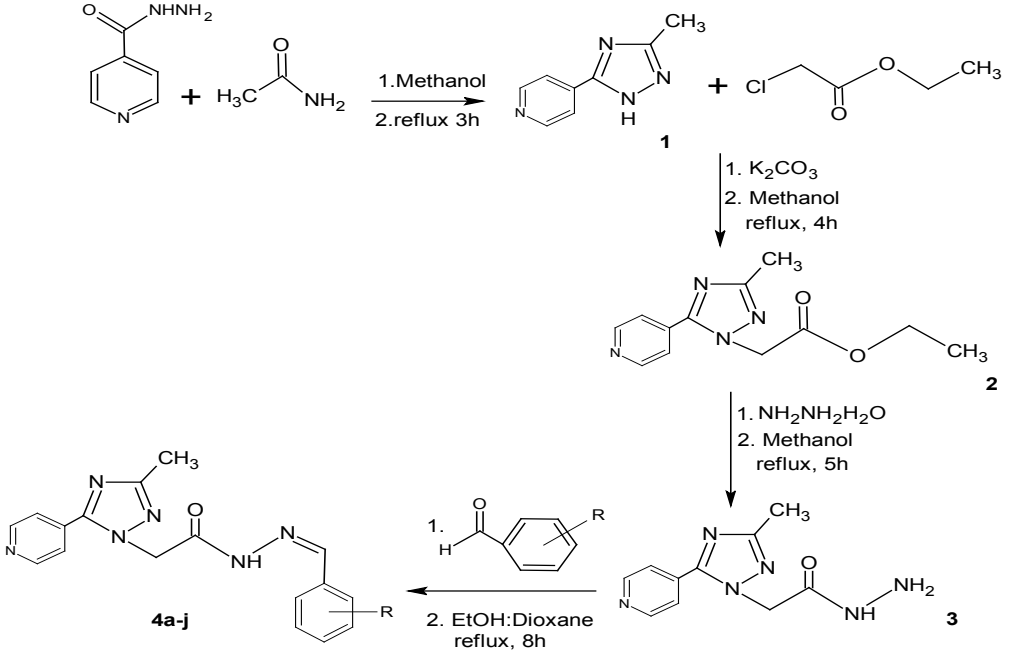
2.2. Antimicrobial activity. The Minimum Inhibitory Concentration (MIC) of the test compounds against the Gram positive *Staphylococcus aureus* (NCIM 2079) and Gram negative *Escherichia coli* (NCIM 2065) bacterial, *Candida albicans* (NCIM 3471) yeast and *Aspergillus niger* (NCIM 1196) mold strains was determined by liquid broth method of two fold serial dilution technique [24]. In this assay, the minimum concentration of each test substance required to inhibit the growth of microorganism was determined. The final concentration of tested compounds ranged from 100 to 12.50 µg/ml. Standard antifungal drug Fluconazole and standard antibacterial drug Ampicillin were tested at concentrations ranging from 100 to 6.25 µg/ml. The tubes were inspected visually to determine the growth of the organism as indicated by turbidity. MIC values of each tested compound are recorded in Table 3.

3. RESULTS SECTION

3.1 Synthesis and spectral characterization. The synthetic strategy for synthesis of the *N*'-[arylidene]-2-(3-methyl-5-pyridin-4-yl-1H-1,2,4-triazol-1-yl) acetohydrazide was found to be efficient with good reaction yield and purity of compounds. The structures of target compounds were assigned on the basis of different physical and spectral studies. The physical data, Fourier transform

infrared spectroscopy (FTIR), ^{1}H-NMR, mass spectral data and elemental analysis of synthesized compounds are reported in experimental protocol (Table 1 and 2).

The melting points of compounds were determined by open tube capillary using Thermonik precision apparatus in Celsius scale and uncorrected. IR spectra were recorded using KBr pellets on PERKIN ELMER 8201 PC IR spectrophotometer, ^{1}H-NMR spectra of the final compound were recorded on BRUKER DRX NMR spectrometer (400 MHz). All spectra were obtained in DMSO. Mass spectra (FAB-MS) of tested compounds were recorded on 70V on JEOL D-300 spectrophotometer (Jeol Ltd., Tokyo, Japan). Elemental analysis for C, H and N were performed on a PERKIN ELMER 240 elemental analyzer.



	R								
4a-j	-H	-2Cl	-4Cl	-4CH_3	-3OCH_3	-4OCH_3	-2NO_2	-4NO_2	-4Br	-4$N(CH_3)_2$

Scheme 1: Synthesis of novel Schiff bases of 1,2,4-triazole (compounds 4a-j).

The synthetic reactions leading to the N'-[arylidene]-2-(3-methyl-5-pyridin-4-yl-1H-1,2,4-triazol-1-yl)acetohydrazide are outlined in Scheme 1. 2-[3-methyl-5-(pyridin-4-yl)-1H-1,2,4-triazol-1-yl]acetohydrazide (2) is the key intermediate for the production of compound 4a-j. Reaction of 4-(3-methyl-1H-1,2,4-triazol-5-yl)pyridine (1) with ethyl chloroacetate in methanol in presence of anhydrous potassium carbonate furnished the ethyl [3-methyl-5-(pyridin-4-yl)-1H-1,2,4-triazol-1-yl]acetate (2) in high yield after treatment with hydrazine hydrate in absolute methanol, it resulted in the formation of the required hydrazide (3). Finally, the title compounds (4a-j) were prepared in great yields by the condensation of hydrazide (3) with different substituted aromatic aldehydes in ethanol:dioxane mixture, using hydrochloric acid as a catalyst. The FTIR spectra of Schiff bases exhibited very similar features and showed the expected bands for the characteristic groups which were present in the compounds. Compounds of 4a-j have C=O stretching bands in the range of 1650-1659 cm^{-1}. Infra red spectrum of compound 4a-j showed a sharp absorption at 1564-1569 cm^{-1}, 782-789 cm^{-1}, 1164-1166 cm^{-1}, 3156 and 695 cm^{-1} which is attributed to -NO_2, -Cl, -OCH_3, -N-(CH_3)2,

and -Br group respectively. Imine group (-N=CH-) also showed characteristic absorption band in range 1622-1629 cm^{-1}. In the ^{1}H-NMR spectral data, all protons were seen according to the expected chemical shift and integral values. The aromatic protons appeared as multiplet peaks within the range of 6.80-7.98 ppm. The ^{1}H-NMR spectra of compounds 4a-j displayed singlet of –NH– groups at 9.22-9.28 ppm while each signal showed integration for one proton. One singlet of -N=CH- group were observed at 7.88-7.97 ppm. Mass spectra of the compounds 4a-j showed molecular ion peaks with high abundance at m/z in agreement with their molecular formula.

Table1: Physical properties of compounds 4a-j.

Comp. code	R	M.P. (°C)	Yield (%)	Mol. form	Mol. Wt	C,H,N Calculated (Found)		
						C%	H%	N%
4a	H	192-194	77	$C_{17}H_{16}N_6O$	320.34	63.74 (63.79)	5.03 (5.09)	26.23 (26.32)
4b	2-Cl	203-205	76	$C_{17}H_{15}ClN_6O$	354.79	57.55 (57.60)	4.26 (4.19)	23.69 (23.62)
4c	4-Cl	207-209	82	$C_{17}H_{15}ClN_6O$	354.79	57.55 (57.49)	4.26 (4.26)	23.69 (23.79)
4d	4-CH_3	228-230	67	$C_{18}H_{18}N_6O$	334.37	64.66 (64.56)	5.43 (5.41)	25.13 (25.10)
4e	3-OCH_3	239-241	71	$C_{18}H_{18}N_6O_2$	350.37	61.70 (61.75)	5.18 (5.19)	23.99 (23.94)
4f	4-OCH_3	242-244	75	$C_{18}H_{18}N_6O_2$	350.37	61.70 (61.62)	5.18 (5.21)	23.99 (23.89)
4g	2-NO_2	196-198	77	$C_{17}H_{15}N_7O_3$	365.34	55.89 (55.81)	4.14 (4.19)	26.84 (26.80)
4h	4-NO_2	199-201	68	$C_{17}H_{15}N_7O_3$	365.34	55.89 (55.80)	4.14 (4.14)	26.84 (26.73)
4i	4-Br	245-247	72	$C_{17}H_{15}BrN_6O$	399.24	51.14 (51.19)	3.79 (3.71)	21.05 (21.11)
4j	4-N- $(CH3)_2$	207-209	84	$C_{19}H_{21}N_7O$	363.41	62.79 (62.70)	5.82 (5.89)	26.98 (26.92)

Table 2: Spectral data of compounds 4a-j.

Comp. code	R	IR (KBr): cm^{-1}	^{1}H-NMR (400 MHz DMSO):δ ppm	MS m/z (M^+)
4a	H	3435(-NH), 3059(Ar-CH), 2375, 2247(-NCH_2), 1651 (-CO), 1628(-N=CH-)	9.25 (s, 1H, NH), 7.93 (s, 1H, N=CH), 7.90- 6.80 (m, 9H, Ar), 5.57 (s, 2H, -CH_2), 2.20 (s, 3H, triazole CH_3)	320
4b	2-Cl	3437(-NH), 3054(Ar-CH), 2379, 2241(-NCH_2), 1659 (-CO),1625(N=CH), 782 (C-Cl)	9.27 (s, 1H, NH), 7.91 (s, 1H, N=CH), 7.93- 6.80 (m, 8H, Ar), 5.55 (s, 2H, -CH_2), 2.22 (s, 3H, triazole CH_3)	354
4c	4-Cl	3431(-NH), 3049(Ar-CH), 2381, 2248(-NCH_2), 1652 (-CO), 1627(-N=CH-), 789(C-Cl)	9.24 (s, 1H, NH), 7.91 (s, 1H, N=CH), 7.90- 6.82 (m, 8H, Ar), 5.54 (s, 2H, -CH_2), 2.25 (s, 3H, triazole CH_3)	354
4d	4-CH_3	3433(-NH), 3049(Ar-CH), 2378, 2249(-NCH_2), 1657 (-CO), 1624(-N=CH-)	9.28 (s, 1H, NH), 7.95(s, 1H, N=CH), 7.97- 6.85 (m, 8H, Ar), 5.52 (s, 2H, -CH_2), 2.21 (s, 3H, triazole CH_3), 2.88 (s, 3H, CH_3)	334

4e	3-OCH_3	3434(-NH), 3045(Ar-CH), 2372, 2242(-NCH_2),1655 (-CO), 1622(-N=CH), 1164(-OCH_3)	9.27 (s, 1H, NH), 7.96(s, 1H, N=CH), 7.89- 6.80 (m, 8H, Ar), 5.53 (s, 2H, -CH_2), 2.21 (s, 3H, triazole CH_3), 4.04(s, 3H, -OCH_3)	350
4f	4-OCH_3	3431(-NH), 3054(Ar-CH), 2375, 2246(-NCH_2),1657 (-CO), 1624(-N=CH-),1166 (-OCH_3)	9.25 (s, 1H, NH), 7.97(s, 1H, N=CH), 7.91- 6.86 (m, 8H, Ar), 5.51(s, 2H, -CH_2), 2.23 (s, 3H, triazole CH_3), 4.02(s, 3H,-OCH_3)	350
4g	2-NO_2	3430(-NH), 3059(Ar-CH), 2371, 2243(-NCH_2), 1650 (-CO), 1629(-N=CH-),1564 (-NO_2)	9.25 (s, 1H, NH), 7.93(s, 1H, N=CH), 7.90- 6.87 (m, 8H, Ar), 5.50(s, 2H, -CH_2), 2.27 (s, 3H, triazole CH_3)	365
4h	4-NO_2	3435(-NH), 3057(Ar-CH), 2374, 2249(-NCH_2), 1651 (-CO), 1622(-N=CH-), 1569(-NO_2)	9.26 (s, 1H, NH), 7.96(s, 1H, N=CH), 7.93-6.84 (m, 8H, Ar), 5.54(s, 2H, -CH_2), 2.27 (s, 3H, triazole CH_3)	365
4i	4-Br	3439(-NH), 3052(Ar-CH), 2373, 2240(-NCH_2), 1658 (-CO), 1624(-N=CH-), 695(C-Br)	9.22 (s, 1H, NH), 7.88(s, 1H, N=CH), 7.96-6.80 (m, 8H, Ar), 5.51(s, 2H, -CH_2), 2.24(s, 3H, triazole CH_3)	399
4j	4-N-$(CH3)_2$-	3431(-NH), 3054(Ar-CH), 3156(-N(CH_3)2), 2376, 2244(-NCH_2), 1658 (-CO), 1624(-N=CH-)	9.22 (s, 1H, NH), 7.90(s, 1H, N=CH), 7.98-6.81 (m, 8H, Ar), 5.54(s, 2H, -CH_2), 2.21 (s, 3H, triazole CH_3), 2.44 (s, 6H, -N(CH_3)2)	363

3.2 Antibacterial and antifungal activity. The antibacterial and antifungal activity data of the tested compounds are given in Table 3. Significant inhibitory activity at MIC=25/50 µg/mL was observed against the studied pathogenic bacteria. *Staphylococcus aureus* was the most sensitive strain. Most of the compounds had remarkable antifungal activity against the microorganisms employed in this study. Seven out of ten studied compounds exhibited a good antifungal activity (MIC=25/ 50 µg/mL) against both *Candida albicans* and *Aspergillus niger*. As it can be seen in Table 3, *Aspergillus niger* was more susceptible than *Candida albicans* to the studied compounds. From the outcome of antibacterial screening, it is apparent that most of the compounds are possessing antibacterial potential against *Staphylococcus aureus* and *Escherichia coli*. The compounds 4c, 4h, 4b, 4d, and 4e possess very good antibacterial activity with MIC values ranging from 25 to 50 µg/mL. The compounds 4a, 4g and 4i showed moderate antimicrobial activity. The compound 4j showed weak activity with a MIC of 100 and 75 µg/mL. The antifungal screening data revealed that the compounds 4b and 4h showed significant antifungal potential with MIC of 25/50 µg/mL respectively. The compounds 4c, 4d, 4f and 4h exhibited good antifungal activity against *Candida albicans* and *Aspergillus niger*. The compounds 4i and 4j showed moderate activity against the tested fungi. The compound 4a was found to be the weakest antifungal agent from the newly synthesized series with a MIC of 100 µg/mL.

Table 3: Antimicrobial activity of the compounds 4a-j.

Tested compound	**Minimum inhibitory concentration expressed in µg/mL**			
	S. aureus	***E. coli***	***C. albicans***	***A. niger***
4a	75	75	100	100
4b	25	50	25	25
4c	25	25	25	50

4d	25	50	50	25
4e	50	25	25	50
4f	50	50	50	25
4g	50	50	25	25
4h	25	25	50	25
4i	75	50	100	75
4j	100	75	100	75
Ampicillin	12.5	12.5	-	-
Fluconazole	-	-	12.5	12.5

4. CONCLUSIONS

Various substituted *N*'-[arylidene]-2-(3-methyl-5-pyridin-4-yl-1H-1,2,4-triazol-1-yl)acetohydrazide derivatives (4a-j) were synthesized, characterized and screened for their potential antimicrobial activities in comparison with the standard drug Ampicillin and Fluconazole. The compounds 4b, 4c and 4h exhibited the most intensive and consistent antimicrobial activity.

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