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Synthesis and Antimicrobial Activities of Some 5-(4'-Pyridyl)-4-Substituted Benzylideneamino-3-Mercapto(4H)-1,2,4-Triazoles

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Certain 5-(4'-pyridyl)-4-substituted benzylideneamino-3-mercapto(4H)-1,2,4-triazoles having different substitutions on the aromatic ring possessing azomethine linkage were synthesized and evaluated for their antimicrobial activities. The compounds with a 4-dimethylamino, 3,4-dimethoxy, 2-nitro and 4-chloro group on the aromatic ring showed good antimicrobial activity.

Triazoles[1-5] have been reported to exhibit significant antibacterial and antifungal activities. Itraconazole is a well established triazole antifungal agent. Antimicrobial activity of 1,2,4-Triazoles having mercapto[6,7] or mercapto acetic acid[8] at different position have been reported. Compounds with azomethine linkage[9] were also shown to possess good antimicrobial activity. In view of these observations it was thought of interest to synthesize some new 5-(4'-pyridyl)-4-substituted benzylideneamino-3-mercapto(4H)-1,2,4-triazoles having an azomethine linkage. Isonicotinic acid hydrazide which is also a well established antitubercular drug has been incorporated with a view to enhance the activity of triazole derivatives (scheme 1).

All melting points were determined in open glass capillaries and are uncorrected. The purity of the compounds were ascertained by TLC on silica gel-G plates using the solvent systems; benzene:acetone (8:2), toluene:ethylacetate:formic acid (5:4:1) and iodine vapour as detecting agent. IR spectra were taken using the KBr disc technique on a Jasco FTIR 410 Spectrophotometer. ^{1}H NMR spectra were recorded on a Brucker DRX-300 NMR Spectrometer in $CDCl_3$ and DMSO-d_6 with TMS as internal standard. The mass spectra were recorded on a Jeol SX 102 (FAB) mass spectrometer. All the chemicals used were of LR and AR grade and was procured from S. D. Fine Chem. Ltd., New Delhi, E. Merck, Delhi and Central Drug House Pvt. Ltd., Delhi. Potassium isonicotinoyl dithiocarbazinate (1) was prepared using a method that has been reported in the literature[10].

For the synthesis of 5-(4'-pyridyl)-4-amino-3-mercapto(4H)-1,2,4-triazole (2), potassium dithiocarbazinate (0.02 mol) was dissolved in water (5 ml). To this solution, 99% hydrazine hydrate (0.04 mol) was added. The reaction mixture was refluxed on a water bath until the evolution of H_2S gas ceased. It was then diluted with water (20 ml) and carefully acidified with glacial acetic acid. The colourless solid thus separated was filtered, washed with water, dried

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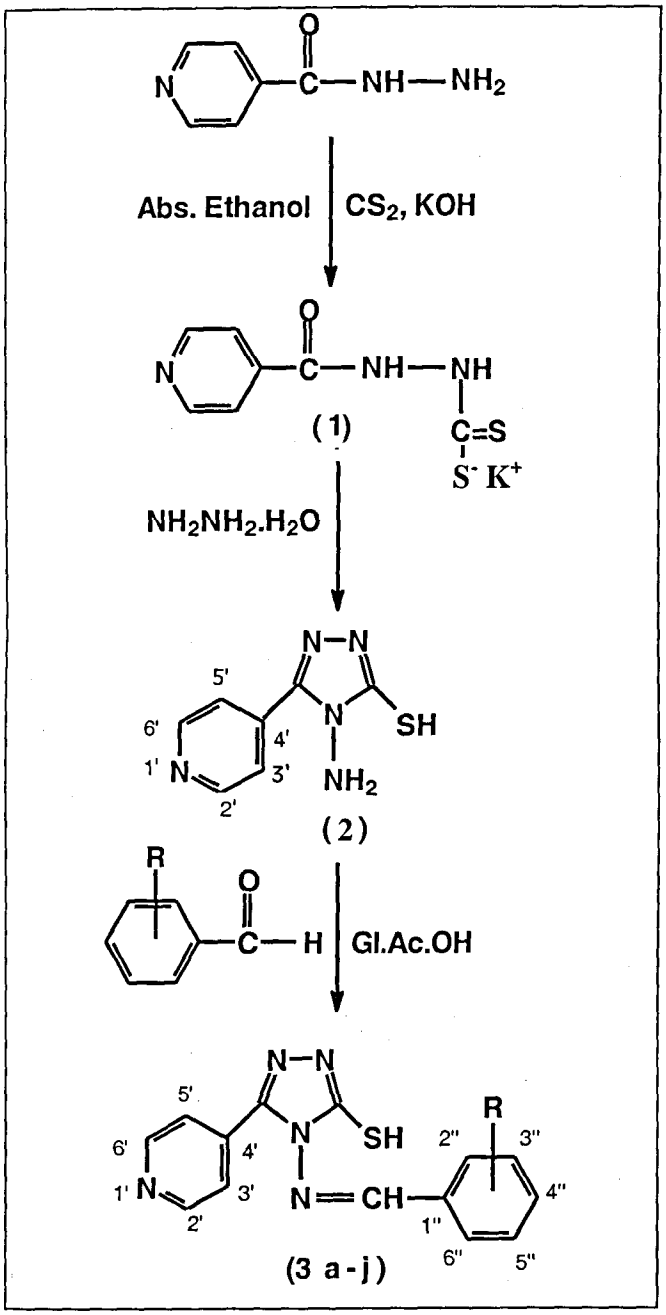

Scheme 1: Synthesis of Benzylideneamino mercapto triazoles

and recrystallized from ethanol, mp: 206°, yield: 87%, ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 5.86 (s, 2H, NH_2), 8.00-8.08 (dd, 2H, 3',5'-ArH), 8.74-8.81 (dd, 2H, 2',6'-ArH), 14.18 (s, 1H, SH); FAB-MS (m/z): 193, 194, 120, 105.

The General procedure used for the preparation of 5-(4'-pyridyl)-4-substituted benzylideneamino-3-mercapto(4H)-1,2,4-triazoles (3a-j) is as follows; a mixture of 2 (0.05 mol) and substituted aromatic aldehyde (0.05 mol) in glacial acetic acid (30 ml) was refluxed for 6 h. The reaction mixture was cooled and poured over crushed ice. The solid thus obtained was filtered, washed with water and recrystallized from glacial acetic acid (Table 1). (3a): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.02 [s, 6H, $N(CH_3)_2$], 6.79-6.81 (d, 2H, 2",6"-ArH), 7.69-7.72 (d, 2H, 3",5"-ArH), 7.85-7.87 (d, 2H, 3',5'-ArH), 8.71-8.72 (d, 2H, 2',6'-ArH), 9.21 (s, 1H, N=CH), 14.33 (s, 1H, SH); FAB-MS (m/z): 324, 325, 244, 179, 147, 120; (3b): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.76 (s, 3H, 4-OCH_3), 3.85 (s, 6H, 3,5-OCH_3), 7.24 (s, 2H, 2",6"-ArH), 7.86-7.88 (d, 2H, 3',5'-ArH), 8.75-8.77 (d, 2H, 2',6'-ArH), 9.64 (s, 1H, N=CH), 14.45 (s, 1H, SH); (3c): IR (KBr, υ_{max} in cm^{-1}): 2990 (CH), 1514 (CN), 1277 (C=S); (3d): ^{1}H NMR (300 MHz, DMSO-d_6) ä ppm: 3.85 (s, 3H, OCH_3), 7.11-7.14 (d, 2H, 3',5'-ArH), 7.85-7.90 (dd, 4H, 2",3",5",6"-ArH), 8.73-8.75 (d, 2H, 2',6'-ArH), 9.52 (s, 1H, N=CH), 14.14 (s, 1H, SH); FAB-MS (m/z): 311, 312, 232, 179, 133, 120, 105; (3e): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 3.84 (s, 3H, OCH_3), 6.93-7.48 (m, 3H, 2",5",6"-ArH), 7.86-7.88 (d, 2H, 3',5'-ArH), 8.74-8.76 (d, 2H, 2',6'-ArH), 9.41 (s, 1H, N=CH), 10.13 (s, 1H, OH), 14.40 (s, 1H, SH); (3f): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 6.89-7.60 (complex m, 4H, 3",4",5",6"-ArH), 7.87-7.89 (d, 2H, 3',5'-ArH), 8.74 (bs, 2H, 2',6'-ArH), 9.95 (s, 1H, N=CH), 10.50 (s, 1H, OH), 14.41 (s, 1H, SH); (3g): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.38-8.92 (complex m, 9H, ArH), 9.75 (s, 1H, N=CH), 14.50 (s, 1H, SH); (3h): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.76-8.99 (complex m, 8H, ArH), 10.56 (s, 1H, N=CH), 14.55 (s, 1H, SH); (3i): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.63-7.66 (d, 2H, 2",6"-ArH), 7.85-7.86 (d, 2H, 3',5'-ArH), 7.93-7.96 (d, 2H, 3",5"-ArH), 8.74-8.76 (d, 2H, 2',6'-ArH), 9.79 (s, 1H, N=CH), 14.49 (s, 1H, SH); (3j): ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 7.50-7.65 (m, 3H, 4",5",6"-ArH), 7.86-7.88 (d, 2H, 3',5'-ArH), 8.11-8.13 (d, 1H, 3"-ArH), 8.76-8.78 (d, 2H, 2',6'-ArH), 10.47 (s, 1H, N=CH), 14.45 (s, 1H, SH).

All the compounds have been screened for both antibacterial and antifungal activity using cup-plate agar diffusion method[11] by measuring the inhibition zone in mm. Amikacin (100 µg/ml) was used as a standard for antibacterial activity and fluconazole (100 µg/ml) as a standard for antifungal activity. The compounds were screened for antibacterial activity against *Escherichia coli* (ATCC 25922) in nutrient agar medium and for antifungal activity against *Candida albicans* (ATCC 2091) in Sabouraud's dextrose agar medium. These sterilized agar media were poured into petri

TABLE 1: PHYSICAL DATA OF 1,2,4-TRIAZOLE DERIVATIVES.

Compound No.	R	mp (°)	Yield (%)	Molecular formula	Nitrogen %	
					Found	Calcd.
3 a	4-$N(CH_3)_2$	332	70.8	$C_{16}H_{16}N_6S$	26.64	25.92
3 b	3,4,5-OCH_3	282	82.0	$C_{17}H_{17}N_5O_3S$	18.53	18.86
3 c	3,4-OCH_3	286	69.8	$C_{16}H_{15}N_5O_2S$	20.17	20.52
3 d	4-OCH_3	316	69.0	$C_{15}H_{13}N_5OS$	22.81	22.50
3 e	4-OH, 3-OCH_3	294	71.8	$C_{15}H_{13}N_5O_2S$	21.12	21.40
3 f	2-OH	232	56.7	$C_{14}H_{11}N_5OS$	23.19	23.56
3 g	H	252	61.4	$C_{14}H_{11}N_5S$	25.27	24.91
3 h	2-NO_2	262	90.6	$C_{14}H_{10}N_6O_2S$	25.47	25.76
3 i	4-Cl	322	81.0	$C_{14}H_{10}N_5SCl$	22.43	22.18
3 j	2-Cl	328	61.0	$C_{14}H_{10}N_5SCl$	22.69	22.18

dishes and allowed to solidify. On the surface of the media microbial suspensions were spread with the help of sterilized triangular loop. A stainless steel cylinder of 8 mm diameter (pre-sterilized) was used to bore the cavities. All the synthesized compounds (100 ìg/ml) were placed serially in the cavities with the help of micropipette and allowed to diffuse for one h. DMF was used as a solvent for all the compounds and as a control. These plates were incubated at 37º for 24 h and 28º for 48 h for antibacterial and antifungal

TABLE 2: ANTIMICROBIAL ACTIVITIES OF 1,2,4-TRIAZOLE DERIVATIVES.

Compound	Antibacterial activity		Antifungal activity	
	Zone of inhibition (mm)	% inhibition	Zone of inhibition (mm)	% inhibition
3 a	15	78.9	22	84.6
3 b	11	57.9	23	88.5
3 c	14	73.7	24	92.3
3 d	10	52.6	21	80.8
3 e	09	47.4	24	92.3
3 f	08	42.1	22	84.6
3 g	05	26.3	25	96.2
3 h	08	42.1	22	84.6
3 i	10	52.6	26	100
3 j	06	31.6	25	96.2
Amikacin	19	100	-	-
Fluconazole	-	-	26	100

Test organisms used were *E. coli* (antibacterial) and *Candida albicans* (antifungal). Concentration of test compounds and standard used was 100 µg/ml.

activities, respectively. The zone of inhibition observed around the cups after respective incubation was measured and % inhibition of the compounds were calculated. The results are presented in Table 2.

Antibacterial activity was shown by all test compounds and the activity ranged from 26.3 to 78.9%. The minimum activity was shown by the compound 3g (26.3%) having an unsubstituted benzylideneamino group. When the substitution was made in the benzylideneamino group activity started increasing. Benzylideneamino group having substitution at p-position by chloro (52.6%), methoxy (52.6%) and N,N-dimethylamino (78.9%) groups showed an increase in the activity in comparison to o-substitution by chloro (31.6%), nitro and hydroxy (42.1%) groups. It was interesting to note that 3,4-dimethoxy substitution in the benzylideneamino group showed more activity (73.7%) than their corresponding 3,4,5-trimethoxy (57.9%) and 4-methoxy (52.6%) substituents. Thus it was concluded that among all 1,2,4-triazole derivatives antibacterial activity decreases when there is o-substitution and it increases with p-substitution showing maximum activity by N,N-dimethylamino group attached to 4th position of the benzylideneamino group (78.4%).

The results of antifungal activity of the test compounds (3a-j) were found to be quite different from their antibacterial activity. All compounds showed significant activity ranging from 80.8 to 100%. The compound (3g) having an unsubstituted benzylideneamino group showed 96.2% activity. Substitution at 2 position by a chloro group retained the activity whereas substitution by 4-chloro group resulted in an increase in activity and it was found to be equivalent to standard drug fluconazole (100%). Substitution in the benzylideneamino group by 2-nitro, 2-hydroxy and N,N-dimethylamino group decreases the activity to the same extent (84.6%). It was noted that 3,4-dimethoxy substituent showed more activity (92.3%) than their corresponding 3,4,5-trimethoxy (88.5%) and 4-methoxy (80.6%) substituents. The compound 3e having 4-hydroxy,3-methoxy substitution showed activity equivalent to the compound 3c having 3,4-dimethoxy substitution (92.3%). Thus it was observed that the compound having p-chloro substituent is the most potent followed by o-chloro, unsubstituted benzylideneamino group, 3,4-dimethoxy and 4-hydroxy, 3-methoxy substituents.

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