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Synthesis and Antimicrobial Evaluation of some newer symmetrical 1, 4-Dihydropyridines.

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Abstract: Bacterial resistance is a major drawback in chemotherapy of infectious diseases. In this investigation a new series of 4-(5'-chloro-3'-methyl-1'-N-phenyl-pyrazol4'yl)-2,6-dimethyl-3,5-disubstituted phenyl- carbamoyl-1,4-dihydropyridines (I_{a-t}) have been synthesized by the cyclocondensation of 5-chloro-3-methyl-1-phenyl-pyrazole-4-carba- ldehyde and N-substituted phenyl)-3-oxobutanamides and ammonia and studied for their enhancing effect on the antibacterial activities towards S. pyogens MTCC-443, S. aureus MTCC-96 and P. aeruginosa MTCC-441(Gram positive) and E. Coli MTCC-442 (Gram negative) bacterial strains and anti fungal activity towards Aspergillus niger MTCC-282 and A. clavatus MTCC-1323 at different concentration for their MIC values using disc diffusion method. During preliminary screening, compounds I_c,I_e,I_g,I_j showed the most enhancing effect on the antibacterial activity as compare to standard drug amoxicillin.The structures of title compounds were elucidated by the IR, NMR and Mass spectrometry and further confirmed by Elemental analyses.
Keywords: Pyrazolyl aldehyde, 4-(5'-chloro-3'-methyl-1'-N-phenyl-pyrazol4'yl)-2,6-dime-thyl-3,5-disubstituted phenyl- carbamoyl-1,4-dihydropyridines, Antimicrobial activity.

Introduction:

1,4-Dihydropyridines (DHPs) are class of nitrogen containing heterocycles having a 6-membered ring. 1,4-DHPs, which are the most potent calcium antagonists or calcium channel blockers, have received much attention due to their wide range of pharmaceutical and biological properties such as inhibition of human cytochrome P450 enzyme, [1] angiotensine-converting enzyme inhibition, and blood pressure control on chronic, nondiabetic nephropathies.[2] 1,4-DHP compounds play important roles in medicinal chemistry, for example nifedipine, amlodipine, felodipine, and nicardipine, which are the best selling drugs used in the treatment of cardiovascular diseases. [3]

One of the major problems in community is the emergence and spread of MDR organisms such as *Staphylococcus aureus* that display resistance to methicillin; cloxacillin and other narrow-spectrum beta-lactamase-resistant penicillin antibiotics as well as cephalosporins [4,5]. These bacteria are labeled as methicillin-resistant *Staphylococcus aureus* (MRSA). The hospital MRSA is usually multi-drug resistant, demonstrating resistant to other classes of antibiotics. Overuse of antibiotics and the use of improper drugs may be some of the reasons for development of virulent strains. Infections with these organisms

represent a serious challenge to the practitioner as therapeutic options are limited and associated mortality is high [6]. Many institutions have observed an increase in blood culture isolates of MRSA over the past two decades.

There is a clear and urgent need to discover and develop new effective and non-toxic drugs that are able to overcome MDR. Some of the medicines have been reported to enhance the antibacterial activity of different antibiotics against different resistant strains [7-9].1,4-Dihydropyridine derivatives have been found as multidrug-resistance (MDR) reversal agents in tumor cells [10]. In continuing investigation on 1,4-dihydropyridine compounds [11-12] and to characterize new synthetic compounds with activity as modulators of MDR in *Staphylococcus aureus* [13].

Generally, 1,4-DHPs are synthesized by the Hantszch method, which involves cyclocondensation of aldehyde, *β*-ketoester, and ammonia or any ammonium sources either in acetic acid at room temperature or refluxing in alcohol for a long time. Some new 4-(5'-chloro-3'-methyl-1'-N-phenyl-pyrazol4'yl)-2,6-dimethyl-3,5-disubstituted phenylcarbamoyl -1,4-dihydro pyridines (I_{a-j}) were synthesized and their antibacterial properties were evaluated.

Materials and Methods

All the melting points were determined routinely in an open capillary tube and are uncorrected. Completion of reaction was routinely checked by TLC on silica gel-G plates of 0.5 mm thickness and the spots were located by iodine. The PMR spectra were recorded in CDCl3 on a Brucker DRX-300 at 300 MHz. The IR spectra were recorded on a Shimadzu-8400 FT-IR spectrometer in KBr. Elemental analyses of the newly synthesized compounds were carried out on a Carlo Erba-1108 analyzer and result within the range of the theoretical value was found. Mass spectra were scanned on a GCMS-QP200 instrument.

General Method of Synthesis of 4-(5'-chloro-3'-methyl-1'-N-phenyl-pyrazol4'yl)-2,6-dimethyl-3,5-disubstituted phenylcarbamoyl-1,4-dihydro pyrimidines (I_{a-j})

(i) Preparation of N-phenyl Oxobutanamide:
The Synthesis of N-phenyl Oxobutanamide[14]

(ii)Preparation of 4-(5'-chloro-3'-methyl-1'-N-phenyl-pyrazol4'yl)-2,6-dimethyl -3,5-disubstituted phenylcarbamoyl-1,4-dihydropyridines (I_{a-j})

A mixture of N-phenyl Oxobutanamide (0.02 M), 5-chloro-3-methyl-1-phenyl-pyrazole-4-carbaldehyde (0.01 M) and ammonia (4-5 ml) in methanol/ DMF (15-20 ml) was heated under reflux condition for 7-10 hrs. The reaction mixture was monitored by TLC by time to time. The reaction mixture was allowed to cool at room temperature/ poured in to water. The solid product was so obtained, was filtered, washed with water, dried and crystallized from either ethanol/ DMF.

The physical data are recorded in **Table no. I.**

Reaction Scheme:

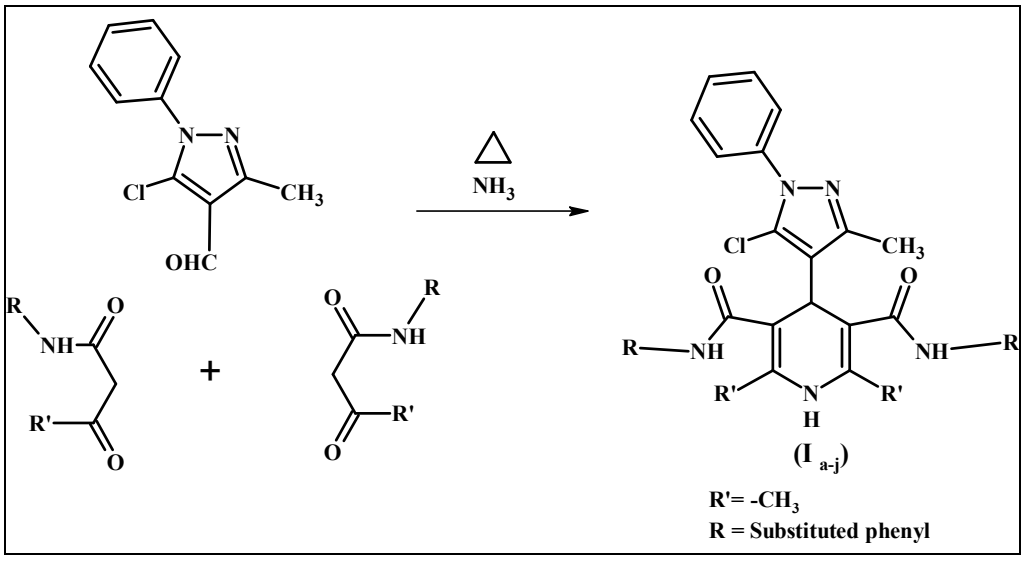

Table No.I: Physical data of 4-(5'-chloro-3'-methyl-1'-N-phenyl-pyrazol4'yl)-2,6-dime- thyl-3,5-disubstituted phenylcarbamoyl-1,4-dihydropyridines (I_{a-j})

Sr. No.	Code	R	R'	MF	MW	MP °C	Yield	Rf
1	Ia	3-CH_3 -C_6H_5	-CH_3	$C_{33}H_{32}N_5O_2Cl$	566.50	211	60	0.40
2	Ib	4-CH_3 -C_6H_5	-CH_3	$C_{33}H_{32}N_5O_2Cl$	566.50	191	59	0.48
3	Ic	2,5-CH_3 -C_6H_5	-CH_3	$C_{35}H_{36}N_5O_2Cl$	594.50	178	65	0.39
4	Id	4-OCH_3 -C_6H_5	-CH_3	$C_{33}H_{32}N_5O_4Cl$	598.50	181	61	0.58
5	Ie	3-Cl -C_6H_5	-CH_3	$C_{31}H_{26}N_5O_2Cl_3$	606.50	216	71	0.38
6	If	4-Cl -C_6H_5	-CH_3	$C_{31}H_{26}N_5O_2Cl_3$	606.50	219	56	0.49
7	Ig	2-F -C_6H_5	-CH_3	$C_{31}H_{26}N_5O_2ClF_2$	574.50	186	75	0.41
8	Ih	4-F -C_6H_5	-CH_3	$C_{31}H_{26}N_5O_2ClF_2$	574.50	185	62	0.61
9	Ii	3-NO_2 -C_6H_5	-CH_3	$C_{31}H_{26}N_7O_6Cl$	628.50	199	74	0.62
10	Ij	4-NO_2 -C_6H_5	-CH_3	$C_{31}H_{26}N_7O_6Cl$	628.50	205	53	052

Spectral analysis4-(5'-chloro-3'-methyl-1'-N-phenyl-pyrazol4'yl)-2,6-dimethyl-3,5-disub stituted phenyl carbamoyl-1,4-dihydropyridines (I_{a-j}):

Comp.	IR (KBr) v(cm-1)	H NMR ($CDCl_3$) □ in ppm
Ia	3363 (-NH), 3012 (Ar-H), 2950 (CH_3), 2894, 1674 (C=O), 1482 (C=C)	1.97 (s,2CH_3), 2.35 (s,CH_3), 3.60 (s,CH_3), 5.10 (s,Ar-H-Pyridine ring), 6.95-7.03 (m,Ar-H), 7.05–7.41 (m-Ar-H), 7.42-7.52 (m,Ar-H), 9.97 (s,-NH);
Ib	3359 (-NH), 3009 (Ar-H), 2957 (-CH_3), 2886, (CH_2), 1680 (C=O), 1485 (C=C)	1.88 (s,2CH_3), 2.42 (s,CH_3), 3.55 (s,CH_3), 5.25 (s,Ar-H-Pyridine), 6.85-6.95 (m,Ar-H), 7.42-7.52 (m,Ar-H), 9.86 (s,NH);
Ic	3360 (-NH), 3010 (Ar-H), 2894, 1680 (C=O), 1486 (C=C)	1.69 (s,2CH_3), 2.28 (s,CH_3), 3.51 (s,CH_3), 5.21 (s,Ar-H-Pyridine ring), 7.15–7.56 (m-Ar-H), 10.07 (s,-NH);
Id	3376 (-NH), 3000 (Ar-H), 2961 (-CH_3), , 1668 (C=O), 1491 (C=C)	1.71 (s,2CH_3), 2.31 (s,CH_3), 3.64 (s,CH_3), 5.19 (s,Ar-H-Pyridine ring), 6.93–7.87 (m-Ar-H), 9.89 (s,-NH);
If	3355 (-NH), 3001 (Ar-H), 1682 (C=O), 1478 (C=C), (C-Cl)	1.78 (s,2CH_3), 2.39 (s,CH_3), 3.72 (s,CH_3), 5.27 (s,Ar-H-Pyridine ring), 6.98–7.76 (m-Ar-H), 9.79 (s,-NH);
Ig	3372 (-NH), 3092 (Ar-H),1669 (C=O), 1489 (C=C), (C-F)	1.69 (s,2CH_3), 2.45 (s,CH_3), 3.85 (s,CH_3), 5.07 (s,Ar-H-Pyridine ring), 6.86–7.91 (m-Ar-H), 9.91 (s,-NH);
Ih	3376 (-NH), 3088 (Ar-H), 2979 (-CH_3), 1671 (C=O), 1480 (C=C),	1.70 (s,2CH_3), 2.30 (s,CH_3), 3.61 (s,CH_3), 5.56 (s,Ar-H-Pyridine ring), 6.90–7.80 (m-Ar-H), 9.79 (s,-NH);,
Ii	3354 (-NH), 3062 (Ar-H), 1662 (C=O), 1472 (C=C), 1521 (Ar-NO_2)	1.81 (s,2CH_3), 2.27 (s,CH_3), 3.79 (s,CH_3), 5.21 (s,Ar-H-Pyridine ring), 7.07-7.58 (m-Ar-H), 10.08 (s,-NH);
Ij	3363 (-NH), 3012 (Ar-H), 2950 (-CH_3), 1674 (C=O), 1482 (C-C), 1112 (C-H);	1.86 (s,2CH_3), 2.34 (s,CH_3), 3.92 (s,CH_3), 5.41 (s,Ar-H-Pyridine ring), 6.97-7.67 (m-Ar-H), 9.78 (s,-NH);

Table No. II: Comparative Antimicrobial Activity of 4-(5'-chloro-3'-methyl-1'-N-phenyl-pyrazol4'yl)-2,6-dimethyl-3,5-disubstituted phenylcarbamoyl-1,4-dihydropyridines (I_{a-j}):

Comp. No.	R	Antibactrial activity (Zones of inhibition in m.m.)									
		S.pyogens MTCC-442					S.aureus MTCC-96				
		5	25	50	100	250	5	25	50	100	250
I_a	3-CH_3-C_6H_4	-	10	15	16	17	-	12	13	15	16
I_b	4-CH_3-C_6H_4	-	12	14	15	18	-	9	13	14	16
I_c	2,5-$(CH_3)_2$-C_6H_3	-	12	13	15	17	-	10	13	15	17
I_d	4-OCH_3-C_6H_4	-	12	15	17	18	-	10	12	14	15
I_e	3-Cl-C_6H_{44}	-	11	13	15	18	-	10	11	15	17
I_f	4-Cl-C_6H_4	-	10	13	15	18	-	10	13	15	17
I_g	2-F-C_6H_4	-	11	14	18	19	-	10	14	17	20
I_h	4-F-C_6H_4	-	12	14	18	20	-	12	15	18	19
I_i	3-NO_2-C_6H_4	-	12	15	17	19	-	11	13	15	20
I_j	4-NO_2-C_6H_4	-	10	12	13	19	-	10	12	18	21
Comparative activity of (I_{a-j}) with known chosen standard drugs											
Standard drug							**Antibacterial activity**				
					I_g I_h	I_g I_h $I_{i,Ij}$			I_g I_h	I_g I_h I_j	I_g I_h I_i I_j
Amplicilline		11	14	16	18	19	10	13	14	16	18
Chloramphenicol		10	13	19	20	20	12	14	19	20	21
Ciprofloxacin		16	19	21	21	22	17	19	21	22	21
Norfloxacin		18	19	20	21	21	19	22	25	26	28

Result and discussion:

Antimicrobial activity

The purified products were screened for its antibacterial activity. The nutrient agar broth prepared by the usual method was inoculated especially with 0.5 ml for 24 hrs. Old subculture of *S.aureus, S.pyogens, E.coli,* and *P.aeruginosa* in separate conical flask at 40- 500C and mixed well by gentle shaking. About 25 ml of the contents of the flask were poured and evenly spread in a petridish (13 cm in diameter) and allowed to set for two hrs. The cups (10 mm in diameter) were formed by the help of borer in agar medium and filled with 0.10 ml (1 mg/ml) solution of a sample in dimethyl formamide. The plates ere incubated at 37°C for 24 hrs and the control was also maintained with 0.1 ml of dimethyl formamide in similar manner, and the zones of inhibition of the bacterial growth are measured in mm diameter and are recorded in Table no. II.

Conclusion:

We have developed newer 1,4-Dihydropyridines by simple conventional method. The synthetic protocol has the inherent potential for future drug synthesis.The1,4-dihydro pyridine derivatives (I_g, I_h, I_i I_j) showed potent antimicrobial activity against various bacterial and fungal strains. The maximum antibacterial and antifungal activity was exhibited by I_g, I_h I_i and I_j. The presence of R = Cl constituent on the phenyl ring along with a variation in solubility plays a significant role in determining the antimicrobial activity of the compounds in comparison to other substituents. The F and NO_2 groups on the aromatic ring enhances the activity while anything at the para position reduces activity. From the results, it is clear that these compounds would be better used in drug development to combat bacterial and fungal infections, and would be better used as pesticides in the future as well.

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