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SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW PYRIDINE DERIVATIVES

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ABSTRACT
*Taking a clue from the biologically potent activities of heterocyclic compounds containing pyridine, the present study has been taken up. With a view to synthesize some biologically active compounds, it has been felt worthwhile to study the synthesis of 4-[4-[{(substituted phenylamino)carbonyl}amino]phenoxy]-Nmethylpyridine-2-carboxamide **(Va-j)**, which was synthesized by the condensation of aminophenoxy-N-methylpyridine-2-carboxamide **(III)** with different arylisocyanates **(a-j)** and were purified all the new compounds including those of intermediates by recystallization or by chromatographic techniques, They were characterised using physical & spectral methods (IR, 1HNMR and MS). Evaluation of the new compounds **(Va-j)** was taken up by screening them against different bacteria (the agar diffusion method) against Bacillus subtilis and Staphylococcus aureus (Gram positive bacteria) and Escherichia coli and Proteus vulgaris (Gram-Negative Bacteria) and fungi (against Candida albicans and Yeast (Saccharomyces cerevisiae)). All the compounds exhibited mild to moderate antibacterial activity. Most significant of them was compound **Va** (R= 4-Cl, 3-CF3) showing greater inhibitory effect against organisms employed, particularly against B. subtilis, S. aureum, E. coli & P. vulgaris with zones inhibition of 16, 15, 16 &15 respectively at a concentration of 200 µg/ml. All the compounds also showed mild to moderate antifungal activity. Again compound Va was found to be the most active antifungal also among all the tested compounds. Compounds Vd (R=CF3), Vb (R=2-Cl), Vc (R=F), Ve (R=2, 4-CH3), and Vf (R=3, 5-CH3) have been found as the next in the order of its antifungal potency.*

KEY WORDS
Pyridine, condensation, antibacterial, antifungal, agar diffusion.

INTRODUCTON

Pyridine is a simple aromatic heterocyclic organic compound with the chemical formula C_5H_5N used as a precursor to agrochemicals and pharmaceuticals, and is also an important solvent and reagent. It is structurally related to benzene, wherein one CH group in the aromatic six-membered ring is replaced by a nitrogen atom. It exists as a colourless liquid with a distinctive, unpleasant fish-like odour. The pyridine ring occurs in many important compounds, including nicotinamides.

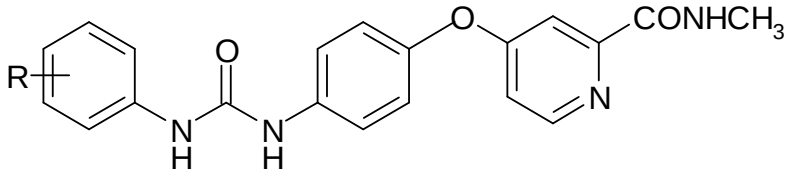
The chemistry of pyridine and its derivatives are well known in the literature. The pyridine chemistry has developed tremendously because of wide applicability to the human kingdom. Pyridine nucleus has been found to have various pharmacological activities like Anticancer, Antibacterial, Antifungal, Anti-inflammatory, Antiulcer, Analgesic, Antihistaminic, Antidepressant, Antiviral and Cardiovascular activities. Chloropyridines feature strongly in the herbicide field and a variety of commercial products exist. For example, Picloram[1], 4-Amino-3,5,6-trichloropicolinic acid, is a potent systemic herbicide usually formulated with 2,4-D or 2,4,5-T. It exhibits a high degree of activity against most broad leaved plants and conifers.

A number of Chloropyridines or derivatives of Chloropyridines exhibit fungicidal or bactericidal activity. 2-Chloro-6-trichloromethylpyridine which is selectively active against Nitrosomonas bacteria, the organism responsible for the conversion or nitrification of ammonium ions to nitrite ions in soil[2]. The most significant benzopyridine fungicides are the normal metal chelate compounds derived from 8-hydroxy quinoline. Related compounds include

quinacetol sulfate and halacrinate which have been used as topical antifungals

Therefore in view of the varied biological & pharmacological properties of pyridine, it has been considered as prime importance to take up such synthesis of 4-substituted N-methylpyridine-2-carboxamide derivatives with a view to get more potent antimicrobial compounds (Figure 1).

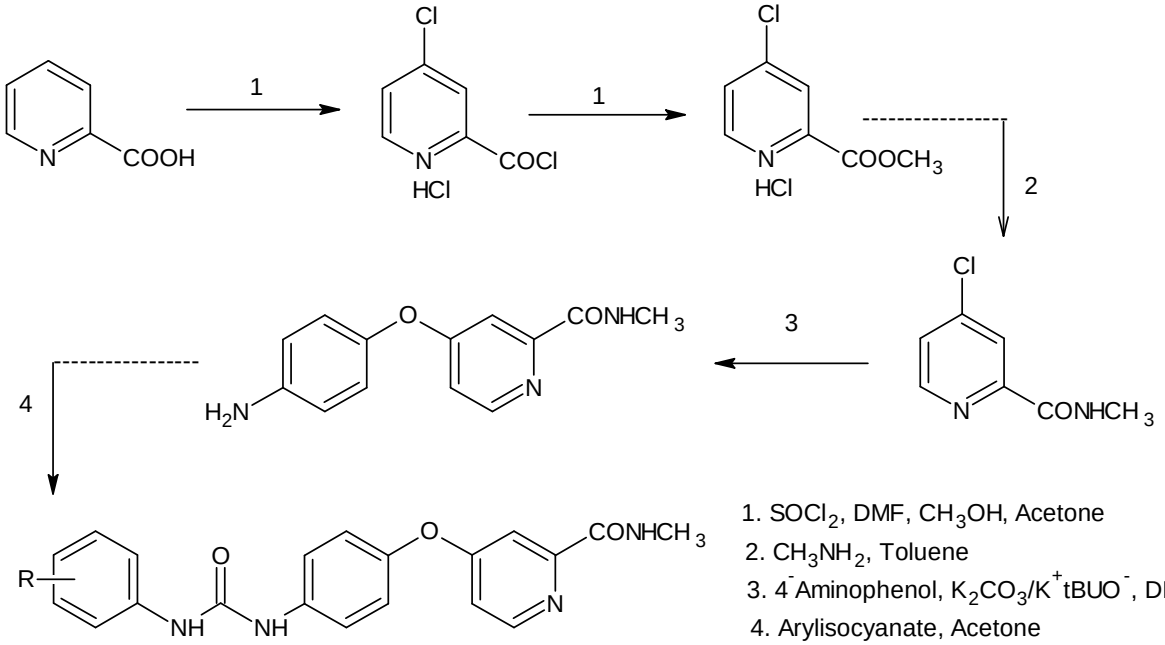
Figure 1: 4-substituted N-methylpyridine-2-carboxamide derivatives



To synthesize new pyridine derivatives by adopting appropriate synthetic routes. Purification & characterization of all the new compounds including those of intermediates by recystallization from appropriate solvents (or) by chromatographic techniques. Characterization of the newly synthesized compounds by physical & spectral methods (IR, ^{1}HNMR and MASS). Evaluation of the new compounds for their possible biological activities like antibacterial and antifungal activities.

In the present investigation, involving reactions of pyridine with a view to synthesize some biologically active compounds, it has been felt worth to study the Synthesis of 4-[4-[{(substituted phenyl amino) carbonyl} amino] phenoxy] – N – methyl pyridine -2-carboxamide. Which is synthesized by the condensation of Aminophenoxy-N-methylpyridine-2-carboxamide with different arylisocyanates, as such reactions are not reported so far, and also to evaluate the products biologically.

SCHEME



1. $SOCl_2$, DMF, CH_3OH, Acetone
2. CH_3NH_2, Toluene
3. 4-Aminophenol, K_2CO_3/K^+tBUO^-, DMF
4. Arylisocyanate, Acetone

EXPERIMENTAL PROCEDURE

Step: 1 (Preparation of Methyl-4-chloropyridinecarboxylate.hydrochloride)

$SOCl_2$ was taken into a 1.0 Litre 4 necked Round Bottomed (RB) Flask. The mass was heated to 40-45°C. DMF was added and stirred the mixture for 15 min. Compound-I was added in lots wise over a period of 30 min and stirred the mixture for 15 min. Then the temperature was raised to 70-75°C. This temperature was maintained for 16 hours. Yellow

solid was formed. Then cool down the solid to RT & diluted with toluene. The remaining $SOCl_2$ was distilled off. Methanol & Toluene was taken into a 1.0 Litre 4necked RB flask. This was cooled to 0-5°C. The reaction mass was added to above mixture. Then solid was formed. This solid was filtered & washed with toluene & chilled acetone.

Step: 2 (Preparation of 4-chloro-N-methylpyridine-2-carboxamide)

Aqueous Monomethylamine & Toluene was taken into a 1.0 Litre 4 necked RB flask. The mass was cooled to 0-5°C. Compound-II was added in lots wise over a period of 30 min and maintained at 0-10°C for 2 hours. TLC was followed. If TLC complies, the RM was extracted with toluene. Carbon treatment and NaCl solution washing was given to the organic layer. Dried and distilled off the toluene.

Step: 3 (Preparation of4-(4'-aminophenoxy)-N-methylpyridine-2-carboxamide)

4-aminophenol & DMF was taken into a 1.0 Litre 4 necked RB flask under Nitrogen. Potassium tertiary butoxide was added and maintained at RT for 2 hours. Compound-III & K_2CO_3 was added and the temperature was raised to 80-85°C. Maintained at 80-85°C upto TLC complies. TLC was followed. The work was proceded if TLC complies. The RM quenched into the water & extracted with toluene. The organic layer was washed with 5% lye solution. Then this was dried & concentrated under vaccum & cooled the residue to -5-0°C for 30-60 min. So that the solid was formed. This solid was filtered and washed with chilled toluene.

Step: 4 (Preparation of 4-(4-{([substitutedphenylamino]carbonyl)amino} phenoxy)-N-methylpyridine-2-carboxamide)

Compound-IV and acetone was taken into a 1.0 Litre 4 necked RB flask. Isocyanate solution (5 gm of isocyanate in 6 ml of acetone) was added slowly. The temperature was maintained to 35°C, So that the solid was formed. This solid was maintained at 30-35°C for 3 hours. The mass was cooled to 10-15°C and maintained for 1 hour. The solid was filtered and washed with chilled acetone.

Procedure for preparation of Isocyanate

Aryl aniline and methylenedichloride was taken into a 1.0 Litre 4 necked RB flask. To this Triphosgene and methylenedichloride was added slowly. The mixture was maintained at room temperature for 2 hours. The mass was heated to reflux. Maintained the reflux up to TLC complies. Further, the work was proceded if TLC complies.

Spectral analysis:

The prepared samples were subjected to spectral analysis by using FTIR and mass spectroscopy.

Biological Activity

In view of varied biological and pharmacological importance of different Pyridines, it has been prompted us to evaluate the new series of 4-[4-[{(substituted phenylamino) carbonyl} amino] phenoxy]-N-methylpyridine-2-carboxamide derivatives (Va-Vj) for antibacterial and antifungal activity.

MATERIALS AND METHODS

Four bacterial test organisms such as *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 722), and *Proteus vulgaris* (MTCC 109) were selected and obtained from the Institute of Microbial Technology, Chandigarh. Cultures of test organisms were maintained on nutrient agar slants and were sub cultured in Petri dishes prior to testing. The media used was nutrient agar, nutrient procured from HiMedia Laboratories, Mumbai. Stock solutions of the synthesized compounds were prepared in the different concentrations, viz., 100µg/ml, 150µg/ml, 200µg/ml using dimethylsulfoxide (DMSO) as solvent for antimicrobial activity.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of title compounds was assayed against four different strains of bacteria by agar diffusion method.

Two Gram-Positive Bacteria: *Bacillus subtilis* and *Staphylococcus aureus*

Two Gram-Negative Bacteria: *Escherichia coli* and *Proteus vulgaris*

Generally, the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar. The bacterial inhibition can be measured by two methods: one is serial dilution method and the other is diffusion

method. The serial dilution method is very useful for the determination of antimicrobial activity. It is not much useful for the quantitative detection tests and for the evaluation of large number of compounds. The cup-plate agar diffusion method was used in this investigation. In this method, cups or discs of standard diameter are made in the nutrient agar medium, containing standard bacterial inoculums. The test compounds were introduced into the discs and the diameter of zone of inhibition was measured.

Antifungal Activity

For the antifungal screening of synthesized compounds, *Candida albicans* and *Yeast* were used. The test organisms were sub cultured using SDA medium. The tubes containing sterilized medium were inoculated with test fungi and after inoculation at 25ºC for 48 hours, were stored at 4ºC in refrigerator. The inoculum was prepared by taking a loopful of stock culture to about 5ml of Sabourad dextrose broth in a test tube. The tubes were incubated at 25ºC for 48 hours before use. The solution of test compound was prepared by a similar procedure described under the antibacterial activity. A reference standard solution of Clotrimazole (10µg/ml) was prepared by dissolving 10mg of Clotrimazole in 10ml of dimethylsulfoxide (DMSO).

The SDA medium was sterilized by autoclaving at 121ºC (15lb/sq.inch) for 15 minutes. The Petri-plates were sterilized in hot-air oven at 160ºC for an hour. Into each sterilized Petri-plate, about 27ml of molten SDA medium was added and incubated at 30ºC for 2 days. After 2 days of incubation, the medium free of contaminations was spreaded with 50µl of 48hours culturing. After solidification of the cups of 6mm diameter were made in each plate with sterile borer. Accurately 50µl of 200µg/ml, 150µg/ml, and 100µg/ml of test solutions were transferred to the respective Petri-plates aseptically and labeled accordingly. The reference standard 50µl was also added to the discs in each plate. The plates were kept in refrigerator for one hour to allow the solution to diffuse properly into the SDA medium. Then, the plates were incubated at 25ºC for 48hours at inverted position. The diameter of zone of inhibition was read with the help of an antibiotic zone reader. The experiment was performed in triplicate.

RESULTS AND DISCUSSION

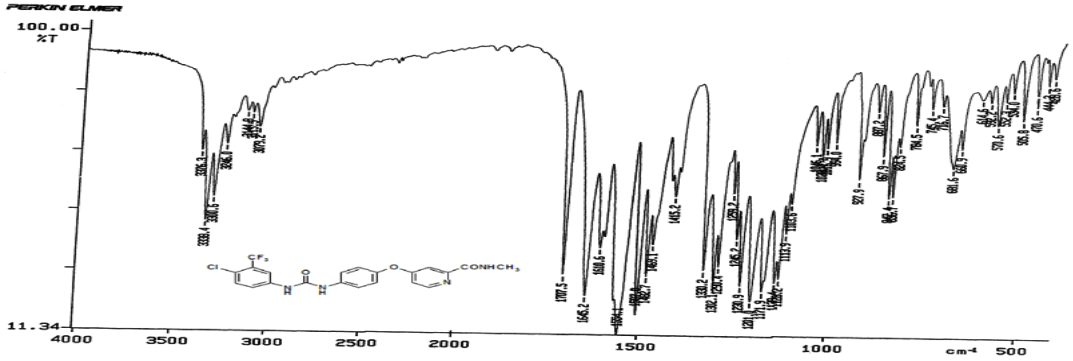
Synthetic work of the study has positively undergone as per the planning and as such in all the reactions carried out, the expected compounds alone could be obtained Physical data of 4-[4-[{(Substituted phenyl amino) carbonyl} amino]phenoxy]-N-methylpyridine-2-carboxamide (V) derivatives was shown in Table 1.

Table 1: Physical data of 4-[4-[{(Substitutedphenylamino)carbonyl}amino]phenoxy]-N-methylpyridine-2-carboxamide derivatives

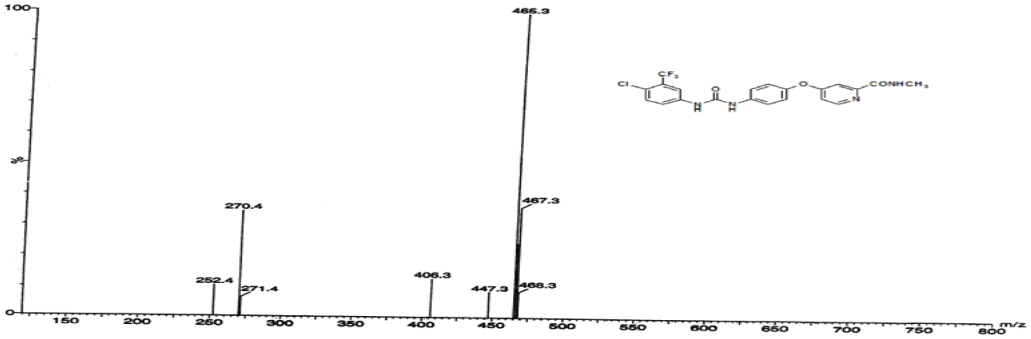
S.No.	Compound	Substituent-R	Mol.formula	Mol.wt.	M.P. (°C)	%Yield
1	Va	4-Cl, 3-CF_3	$C_{21}H_{16}N_4O_3F_3Cl$	464	206-208	89
2	V_b	2-Cl	$C_{20}H_{17}N_4O_3Cl$	396	150-152	97
3	Vc	4-F	$C_{20}H_{17}N_4O_3F$	380	232-234	75
4	V_d	3-CF_3	$C_{21}H_{17}N_4O_3F_3$	430	214-216	81
5	Ve	2,4-CH_3	$C_{22}H_{22}N_4O_3$	390	178-180	78
6	V_f	3,5-CH_3	$C_{22}H_{22}N_4O_3$	390	176-178	80
7	V_g	2-CH_3	$C_{21}H_{20}N_40_3$	376	172-174	85
8	V_h	4-NO_2	$C_{20}H_{17}N_5O_5$	407	226-228	83
9	V_i	3-OCH_3	$C_{21}H_{20}N_4O_4$	392	182-184	80
10	V_J	H	$C_{20}H_{18}N_4O_3$	362	152-154	88

IR (KBr) spectra of V_a compound exhibited 784.5 (C-Cl *str*) 1330.2 (C-F *str*), 1554.1 (Pyridine C=C, C=N, *str*), 1707.5 (C=O *str*), 3079.2 (aromatic, C-H *str*) and 3338.4 (N-H *str*) in Cm^{-1} and Molecular ion (M+1) peak at m/z 465.3 by mass spectra (Figure 2). Vd comppound shows 1342.4 (C-F, *str*), 1570.0 (Pyridine, C=C, C=N, *str*), 1696.0 (C=O, *str*), 3050.5 (C-H-aromatic, *str*) and 3208.2 (N-H, *str*) and mass spectra shows molecular ion (M+1) peak at m/z 431.6 (Figure 3).

Figure 2: IR and mass spectram of 4-[4-[{([4-chloro-3-(trifluoromethyl)phenyl]amino)carbonyl} amino]phenoxy]-N-methylpyridine-2-carboxamide (Va)

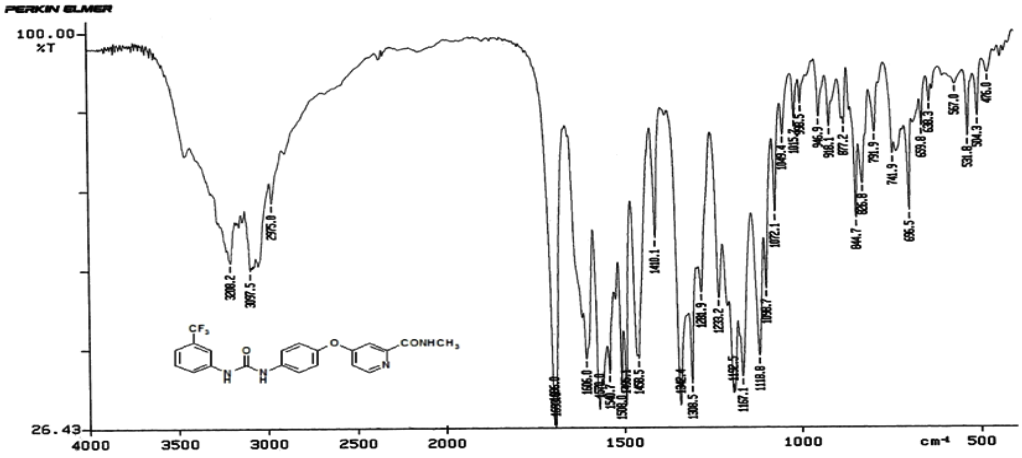


R Spectrum of 4-[4-[{([4-chloro-3-(trifluoromethyl) phenyl] amino) carbonyl} amino] phenoxy]-N-methylpyridine-2-carboxamide. (Va)

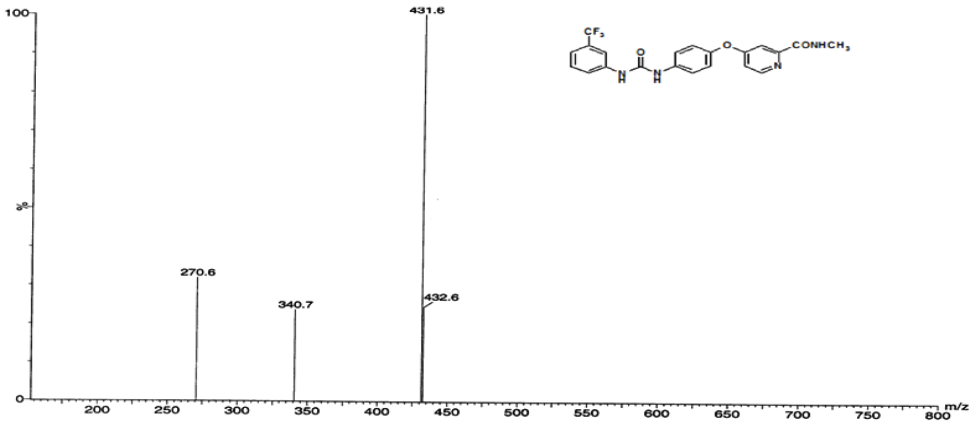


Mass Spectrum of 4-[4-[{([4-chloro-3-(trifluoromethyl) phenyl] amino) carbonyl} amino] phenoxy]-N-methylpyridine-2-carboxamide. (Va)

Figure 3: Spectral data of 4-[4-[{([3-trifluoromethylphenyl]amino)carbonyl}amino] phenoxy]-N-methylpyridine-2-carboxamide (Vd)



IR Spectrum of 4-[4-[{([3-trifluoromethylphenyl] amino) carbonyl} amino] phenoxy]-N-methyl pyridine-2-carboxamide. (v_d)



Mass Spectrum of 4-[4-[{([3-trifluoromethylphenyl] amino) carbonyl} amino] phenoxy]-N-methylpyridine-2-carboxamide.

Antibacterial activity:

The zone of inhibition values of the synthesized compounds against *Bacillus subtilis* and *Staphylococcus aureus* (Gram positive bacteria) and *Escherichia coli* and *Proteus vulgaris* (Gram-Negative Bacteria) were presented in table. Ampicillin was used for the Standard for inhibitory activity against bacteria. All the compounds exhibited mild to moderate activity against bacteria and was shown in table 2. Most significant them has been found to be compound Va with substitutes R= 4-Cl, 3-CF_3 showing greater inhibitory effect against organisms employed, particularly against B.Subtilis, S.aureum, E.coli & P.vulgaris with zones inhibition of 16, 15, 16 &15 respectively at a concentration of 200 µg/ml. This has been followed by compounds Vd (R=CF_3), V_h (R= 4-NO_2), Vb (R= 2-Cl) has been found as the next in order of its antibacterial potency. The compounds Vc(R=F), Ve(R=2, 4-CH_3), and V_f(R=3, 5-CH_3), V_i(R=3-OCH_3), V_j (R=H), V_g (R=2-CH_3) have been moderately potent against all the four strains of bacteria.

Table:2 Antibacterial activity of 4-[4-[{(substituted phenylamino)carbonyl}amino]phenoxy]-N-methylpyridine-2-carboxamide derivatives (Va-j)

S.No	Compound	R	Zone of inhibition (in mm)			
			B.subtilis	S.aureus	E.coli	P.vulgaris
1	Va	4-Cl, 3-CF_3	16	15	16	15
2	Vb	2-Cl	12	12	11	13
3	Vc	4-F	13	14	14	14
4	Vd	3-CF_3	15	15	15	15
5	Ve	2,4-CH_3	9	11	11	12
6	Vf	3,5-CH_3	10	13	11	12
7	V_g	2-CH_3	9	11	10	11
8	V_h	4-NO_2	13	14	15	14
9	V_i	3-OCH_3	11	10	12	10
10	V_J	H	10	11	09	10
Standard drug (Ampicillin)			19	21	20	19

ANTIFUNGAL ACTIVITY

The antifungal activity of the compounds was studied against *Candida albicans* and *Yeast (Saccharomyces cerevisiae).* Clotrimazole was used for the standard for inhibitory activity against fungi. All the compounds showed mild to moderate antifungal activity. Compound Va (R=4-Cl, 3-CF_3) was found to be the most active antifungal agents among all the tested compounds. Compounds V_d(R=CF_3), V_h (R= 4-NO_2), V_b(R=2-Cl), Vc(R=F), Ve(R=2, 4-CH_3), and V_f(R=3, 5-CH_3), V_i(R=3-OCH_3), V_j (R=H), V_g (R=2-CH_3) have been found as the next in the order of its antifungal potency (Table 3).

Table:3 Antifungal activity of 4-[4-[{(substituted phenyl amino) carbonyl} amino]phenoxy]-N-methylpyridine-2-carboxamide derivatives (Va-j).

S.No	Compound	R	Zone of inhibition (in mm)	
			Candida albicans	*Yeast*
1	Va	4-Cl, 3-CF_3	15	14
2	Vb	2-Cl	12	11
3	Vc	4-F	10	11
4	Vd	3-CF_3	13	14
5	Ve	2,4-CH_3	11	10
6	Vf	3,5-CH_3	10	9
7	V_g	2-CH_3	10	9
8	V_h	4-NO_2	14	13
9	V_i	3-OCH_3	12	11
10	V_J	H	10	9
Standard drug (Clotrimazole)			**18**	**19**

CONCLUSION

In the present study new pyridines were synthesized by conventional method as mentioned in the scheme and evaluated for their antimicrobial and antifungal activities. Among the compounds synthesized **Va**, **V**d, Vh and **V**b demonstrated good antibacterial, **Va**, **V**d, Vh and **V**b showed good antifungal activities

respectively in comparision to the control and other test compounds.

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