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EFFECT OF BIOLOGICAL STUDIES OF VARIOUS NEW SUBSTITUTED PHENYL PYRAZOL PYRIDIN-2-AMINE DERIVATIVES

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Effect of Biological Studies of Various New Substituted Phenyl Pyrazol Pyridin-2-Amine Derivatives

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Abstract – A new series of 2-chlorobenzaldehyde N-ethyl pyridin-2-a mine (2a-2e), 1-(5-(2-substitutedphenyl)-3-(pyridin-2-ylamino)-4,5-dihydro-1H-pyrzol-1-yl) ethanone (3a-3e). 2,3-dibromo-3-(2-substitutedphenyl)-N-(pyridin-2-yl) propanamide (4a-4e) & N-(5-(2-substitutedphenyl)-1-phenyl-1H-pyrazol-3-yl) pyridin-2-amine (5a-5e) were prepared in present study. The structure of all these newly synthesized compounds was confirmed on the basis of spectral (IR, ^{1}HNMR and mass) and analytical data. Compound 5b was found to be the most potent compound of the present study. It exhibited better insecticidal and antifungal activities than the standards parathion and fluconazole, respectively,

Keywards: Pyrazoline Derivatives Insecticidal, Antifungal & Antibacterial Activity.

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INTRODUCTION

Imidacloprid, acetamprid (neonicotinoids)[1-2] derivatives of pyridine, act on the central nervous system (CNS) of insects causing irreversible blockage of post synaptic nicotenergic acetylcholine receptor[3] and fipronil a derivative of pyrazole, blocks the γ-aminobutyric acid (GABA) regulated chloride channel in neurons, thereby, antagonizing the calming effects of GABA[4]. It has been found in the literature that pyridine derivatives have been synthesized as insecticidal[5-6] antifungal[7], antibacterial[8], herbicidal agents[9], and substitution pattern of pyridine nucleus at 2-position by different heterocyclic moieties markedly modulates its biological properties. Furthermore, pyrazole and pyrazoline congeners have also been found to exhibit insecticidal[10-13] antifungal[14] antibacterial activities. These findings prompted us to synthesize a new series of pyridine derivatives by adding pyrazole and pyrazoline moieties at its 2-position, with a hope to get better insecticidal potential along with additional biological activities like antifungal and antibacterial.

BIOLOGICAL STUDY

Various compounds have been synthesized and evaluated for insecticidal activity against male or female cockroaches (*Periplaneta americana*). These compounds were also assayed *in vitro* for their antifungal and antibacterial activities.

INSECTICIDAL ACTIVITY

The insecticidal activity was determined by the method of Joshi and Tholia (1973). The cockroaches of either sex were divided in groups having five cockroaches each. An acetone solution (0.02 mL of 5 g/L) of standard insecticide, parathion, and different test compounds were injected on the ventral side of the insect, between the fourth and fifth abdominal segments with the help of a micrometer syringe. Insects receiving 0.02 mL of acetone by the same route served as control.

The treated cockroaches were kept under observation to record the time taken until 100 % mortality. During this period, no food was given. In an other set of experiment, most active compound of each *Series* at two graded doses i.e. 0.2 ml of 10 g/l and 20 g/l were also injected to groups of insects with identical doses of parathion. The statistical significance of the difference between the data of standard and test compounds was calculated by employing student's 't' test (A 1).

ANTIBACTERIAL ACTIVITY

The antibacterial activity of test compounds and standard chloramphenicol was done by filter paper disc method (Gould and Bowie, 1952) against *Staphyloccous aureus 209 p* and *Eschericia coli ESS 2231*, at a concentration of 250 □g/mL. Media with 10% DMSO in methanol was set up as control. The presence of methanol caused no visible change in the bacterial growth. The Whatman filter paper discs of standard size (7 mm) were prepared. These discs were put into 1 oz screw capped wide-mouthed containers. These bottles are then sterilised in hot-air oven at 150 ºC. Solution is then added to each bottle. Before use, the bottles should be shaken to distribute the discs around the walls of the container and this allows them to be picked up more easily with the

forceps. The discs are transferred to the inoculated plates with a pair of fine pointed tweezers. The tweezers may be kept with their tips immersed in 70 % alcohol, which is flamed off before use to prevent contamination.

The test organisms were grown on nutrient agar (A 4) and, before use, were subcultured in nutrient broth at 37 ºC for 18-20 hours. Each disc was applied carefully to the surface of the agar without lateral movement once the surface had been touched; where necessary they were flattened down with the points of the forceps. The plates were then incubated for 24 hours at 37 ºC, and the resulting zones of inhibition (in mm) were measured.

ANTIFUNGAL ACTIVITY

The standard agar disc diffusion method (Pai and Platt, 1995) was performed to evaluate the antifungal property of test compounds and standard fluconazole.

Aspergillus fumigatus, Candida albicans ATCC 2091, Candida albicans ATCC 10231, Candida Krusei GO3 and *Candida glabrata HO5* were used in this study. All cultures were routinely maintained on SDA (A 2) and incubated at 30 ºC. In order to prepare homogeneous suspensions of these fungi for disc assays, they were grown overnight in sabouraud broth, centrifuged to collect the pellet and re-suspended in sterile phosphate buffered saline. The fungal pellet was homogenized in a sterile hand–held homogenizer. This suspension was then plated onto SDA using a bacterial spreader to obtain an even growth field. Sterile 6 mm what man filter paper discs (A 3) were impregnated with 250 □g/mL concentration of various test compounds and standard drug, fluconazole. These discs were then placed in the centre of each quadrant of an SDA plate. Each plate had one control disc impregnated with 10% DMSO in methanol. The plates were incubated at 30 ºC. After 48 hours, the plates were removed and the radius of the zone of inhibition (in mm) was measured.

EXPERIMENTAL

General

All reagents and solvents were generally used as received from the commercial supplier. Reactions were routinely performed in oven-dried glassware. Melting points were determined with an electrothermal melting point apparatus, and are uncorrected.

The homogeneity of all newly synthesized compounds was checked by thin layer chromatography *(*TLC) on silica gel-G coated plates. Eluent was a mixture of different solvents in different proportions, and spots were visualized under iodine chamber. Elemental analysis (C, H, N) of all the compounds was performed on Carlo Erba-1108 elemental analyzer, and results were found within the ± 0.4% of theoretical values.Infrared (IR) spectra (KBr) were recorded on Brucker–IFS–66 FTIR instrument and wave number (□) was recorded in cm^{-1}.^{1}H-NMR spectra were recorded JEOL, GSX-400 FTNMR instrument at 400 MHz in $CDCl_3$ or DMSO-d_6 unless otherwise specified, and chemical shifts (□) are reported in ppm. Relative to tetramethyl silane as an internal standard Mass spectra were determined from Mass Finniganmat 8230 MS.

RESULTS AND DISCUSSION

All the compounds **2a-2e**, **3a-3e, 4a-4e** and **5a-5e** along with reference drug, parathion were assayed for insecticidal activity against *periplaneta americana* at a concentration of 5 g/L. These compounds demonstrated greater level of activity as compared to parathion (Table I). Out of these twenty compounds tested, compound **5b** was found to be most active insecticidal agent. By considering its potentiality, it would be interesting to examine this compound with standard at two more concentrations i.e. 10 g/L and 20 g/L and results given in table I. The fifteen compounds mentioned in insecticidal activity were also screened *in vitro* for antifungal activity at a concentration of 250 □g/ml. Many of these compounds tested produced inhibition growth of different strains of fungi (Table II). Compounds **2a–2e, 3a–3e, 4a–4e** and **5a-5e** were assessed *in vitro* for antibacterial activity against *S. aureus 209 p* and *E. Coli ESS 2231* strains. Some of these compounds were found to exhibit antibacterial activity but not more than the standard drug chloramphenicol (Table II).

Compounds 2a-2e, 3a-3e, 4a-4e and 5a-5e contain following structural features at 2-positon of pyridine nucleus: substituted benzylidene, substituted pyrazoline and substituted pyrazole moieties respectively. Changing the substitution pattern on the phenyl moiety (compounds **2a**, **2b**, **2c**, **2d** and **2e**) affect the insecticidal activity of the compounds in the dose range studied. On comparing the results of **2a-2e** it was notable that compound **2b** with *o*-chlorophenyl substitution was more effective than compound **2a** having *p*-chlorophenyl moiety. Compounds **2c**, **2d** and **2e** substituted with *o*-hydroxy, *p*-methoxy and *p*-aminodimethylphenyl groups, respectively, exhibited moderate activity (214, 245, 233 minutes, respectively). On the basis of above findings, it was observed that chloro group at *o*- or *p*-position of phenyl ring was found to be useful for this activity. Among these, compound **2a**, **2b** and **2c** displayed antifungal activity against the various fungi used except *C. krusei GO3*. On the other hand, compounds **2a** and **2b** showed inhibition against both the bacteria tested, while compounds **2c** and **2d** inhibited the growth of *E. coli ESS 2231*, only. It is tempting to speculate from the above data that compound **2b** gave outstanding control of insects, and inhibiting the growth of fungi and bacteria. Moreover, the effects of pyrazoline and pyrazole rings at 2-position of pyridine nucleus were next examined.

Results indicated that the presence of pyrazoline and pyrazole moieties in compounds **3a-3e** and **5a-5e**, respectively, accelerated insecticidal, antifungal and antibacterial profiles of the compounds as compared to their parent compounds **2a-2e**. However, pyrazole congeners (**5a-5e**) exhibited superiority over pyrazoline derivatives (**3a-3e**) in terms of biological properties. It is pertinent to mention here that the compounds **3a** and **5a** bearing *p*-chlorophenyl group as a substitutent showed appreciable activities, while substitution with *o*-chlorophenyl group as seen in compounds **3b** and **5b** produced more potent insecticidal, antifungal and antibacterial activities. *o*-Hydroxyphenyl substituent in compounds **3c** and **5c** yielded less but still adequate biological profiles. Out of the five pyrazole derivatives (**5a-5e**) examined, compound **5b** was found to be the most potent compound of the present study. It exhibited better insecticidal and antifungal activities than the standards parathion and fluconazole, respectively, and rest compound of the series. It also displayed promising antibacterial activity but not more than standard, chloramphenicol.

Table I: Insecticidal activity of compounds **2a-2e**, **3a-3e, 4a-4e** and **5a-5e** against cockroaches (*periplaneta americana*).

Compou-nds	R'	R	Concentration	Mean killing time (minutes) ± S.E.M.
@Control			0.02 mL	720 ± 10.29
Parathion			5 g/L 10 g/L 20 g/L	280 ± 11.74□□□□□ 247 ± 9.29□□□□ 231 ± 13.75□□□
2a	-	4-ClC_6H_4	5 g/L	204 ± 12.22**
2b	-	2-ClC_6H_4	5 g/L	178.6 ± 6.38***
2c	-	2-OHC_6H_4	5 g/L	214.2 ± 5.80**
2d	-	4-$OCH_3C_6H_4$	5 g/L	245.4 ± 6.37*
2e	-	4-$N(CH_3)_2$ C_6H_4	5 g/L	233.4 ± 7.33**
3a	-	4-ClC_6H_4	5 g/L	175 ± 9.70***
3b	-	2-ClC_6H_4	5 g/L	140 ± 6.33***
3c	-	2-OHC_6H_4	5 g/L	162.6 ± 8.20***
3d	-	4-$OCH_3C_6H_4$	5 g/L	180 ± 11.01***
3e	-	4-$N(CH_3)_2$ C_6H_4	5 g/L	200.6 ± 8.84***
4a	-	4-ClC_6H_4	5 g/L	164.4 ± 5.72**
4b	-	2-ClC_6H_4	5 g/L	132.6 ± 4.80**
4c	-	2-OHC_6H_4	5 g/L	149 ± 6.55**
4d	-	4-$OCH_3C_6H_4$	5 g/L	176.4 ± 9.40***
4e	-	4-$N(CH_3)_2$ C_6H_4	5 g/L	188.2 ± 7.62***
5a	C_6H_5	4-ClC_6H_4	5 g/L	142.4 ± 5.26***
5b	C_6H_5	2-ClC_6H_4	5 g/L 10 g/L 20 g/L	98 ± 7.38*** 67 ± 4.40*** 53 ± 6.23***
5c	C_6H_5	2-OHC_6H_4	5 g/l	139 ± 5.78***
5d	C_6H_5	4-$OCH_3C_6H_4$	5 g/L	166.6 ± 10.71***
5e	C_6H_5	4-$N(CH_3)_2$ C_6H_4	5 g/L	182 ± 6.03***

n = 5 in each group; □□P□ < 0.05, □□□□P < 0.01, □□□□□□P < 0.001 in comparison to control; *P < 0.05, **P < 0.01, ***P < 0.001 in comparison to standard; @acetone.

Table II: Antifungal and antibacterial activities of compounds **2a-2e**, **3a-3e, 4a-4e** and **5a-5e** by filter paper disc and agar diffusion methods, respectively.

Compounds	Antifungal activity# [Diameter of the inhibition zone (mm)]					Antibacterial activity# [Diameter of the inhibition zone (mm)]	
	Aspergillus fumigatus	*Candida albicans ATCC 2091*	*Candida albicans ATCC 10231*	*Candida krusei G03*	*Candida glabrata H05*	*Staphylococcus aureus 209p*	*Escherichia coli ESS 2231*
@Control	0	0	0	0	0	0	0
Fluconazole	0	29	25	19	15	—	—
Chloramp-henicol	—	—	—	—	—	20	20
2a	10	14	11	0	09	08	10
2b	11	12	13	0	10	10	11
2c	08	11	10	0	10	08	10
2d	0	0	0	0	0	0	08
2e	0	0	0	0	0	0	0
3a	12	16	12	10	11	11	12
3b	13	18	15	12	11	13	13
3c	10	12	11	0	10	0	12
3d	0	12	10	0	09	0	10
3e	10	08	08	0	0	10	0
4a	14	17	14	08	12	10	13
4b	15	19	16	0	14	12	14
4c	12	15	10	10	13	0	12
4d	0	13	08	0	10	0	10
4e	8	10	0	12	0	8	0
5a	16	24	20	16	13	14	12
5b	20	33	28	21	17	16	15
5c	15	20	17	15	13	10	12
5d	14	18	13	13	10	0	10
5e	12	13	10	14	15	10	0

#Concentration was 250□g/mL.
@ 10 % DMSO in methanol
— : No activity done.
0 : No inhibition zone.

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