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Synthesis, Characterisation And Screening Of Some New Chlorosubstituted Imidazolo-Pyrazolines With Special Reference To Their Growth Promoting And Curative Impact On *Oyster Mushroom* Crop

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Abstract

Five membered heterocycles with an additional hetero-atom called azoles are well known for their pharmacological, agricultural and industrial applications. The chemotherapeutic agents such as orisul (bacterostatic), antipyrine (antipyretic), butazolidine (anti-inflammatory) contain pyrazoline nucleus. They have remarkable insecticidal activity against the insects like lepidopteran and coleopteran. Imidazole derivatives of pyrazoline substrates were also reported as main constituents of many pesticides used in agriculture. Some of their derivatives show the fungicidal and plant growth regulatory activities. Owing to their applications, a significant amount of research activity has been directed towards synthesis of this class of compounds. In this context, synthesis of some new chlorosubstituted 1-phenyl-3-(2-substitiuted-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chlorophenyl)-imidazolo]-4,5-dihydro-Δ^2-pyrazolines were undertaken from 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-amino-4,5-dihydro-Δ^2-pyrazoline, which was prepared by the reaction of substituted-3-benzoyl-6-chloroflavanone with phenylhydrazine hydrochloride in 1,4-dioxane containing a little piperidine. The structures of newly synthesised compounds were determined on the basis of elemental analysis and spectral characterization. The newly synthesised compounds were assayed for their antimicrobial activity against some fungi viz. Gliocladium roseum (Link) Bainier, Verticillium fungicola and some bacteria viz. Pseudomonas stutzeri, Pseudomonas alcaligens, Pseudomonas fluorescene, Burkholderia gladioli which are mainly responsible for the damage of mushroom crop. So also the titled compounds were screened for their impact on phytotic growth of Oyster mushroom spp.

Keywords: Chlorosubstituted pyrazolines, α-amino ketone of pyrazolines, imidazolo-pyrazolines, acetyl analogues of imidazolo-pyrazolines and antimicrobial activity.

I. INTRODUCTION

Heterocyclic compounds play an important role in mediating many biological processes[1-5]. One of the important reasons for the widespread applicability of heterocyclic compounds is the flexibility of their structure towards modification to incorporate functional moieties either as substituent or as a part of the ring system. Owing to this property, an organic chemist is enabled to tailor a structure to meet a particular need by modifying the heterocyclic component. It is, therefore, not surprising that lot of efforts have been extended in studying their chemistry and applicability.

Literature survey reveals that medicinal as well as agricultural chemists have become interested in the synthesis of pyrazoline analogues possessing active moieties for the development of bioactive agents having greater and improved properties towards respective fields. Kalirajan *et al.*[6] reported the formation of some pyrazoline substituted benzimidazoles and studied their biological activities.

Rajora *et al.*[7] transformed 1-benzimidazolyl-3-aroyl-prop-2-ene-1-one into N-substituted pyrazoline derivatives by the treatment of phenylhydrazine, thiosemicarbazide and hydrazinehydrate in presence of formic acid and reported their antimicrobial properties.

Literature survey also reveals that, mushrooms species easily fall prey to infections caused by pathogens and thus become a serious problem in the mushroom crop cultivation. The diseases like *white cottony growth* and fruiting body covered with the *green spots* are reported to cause by *Gliocladium sp.* In addition to this, diseases like powdery white growth on stipe, fluffy growth on substrate, dry bubble disease, brown spot disease are also reported to be caused by the infection of *Cladobotrym apiculatum, Arthrobotrys pleuroli, Velricillium fungicola* and *Pseudomonas stulzeri* respectively.

Thus it was thought significant to explore the properties of titled compounds against mushroom crop pathogens *viz* fungi *Gliocladium roseum (Link) Bainier, Verticillium fungicola* and bacteria *Pseudomonas stutzeri, Pseudomonas alcaligens, Pseudomonas fluorescene, Burkholderia gladioli* and also their growth promoting and curative impact on *Oyster mushroom* crop.

In tune with the literature survey, we, herein, report the synthesis of 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-amino-4,5-dihydro-Δ^2-pyrazoline (1), 1-phenyl-3-(2-hydroxy-5-chloro phenyl)-4-benzoyl-5-N-[(2'-hydroxy-5'-chlorophenyl)ethanonylamino]-4,5-dihydro-Δ^2-pyrazoline (2), 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chloro phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (3), 1-phenyl-3-(2-acetyloxy-5-chlorophenyl)-4-benzoyl -5-[2-mercapto-4-(2'-acetyloxy-5'-chloro-phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (4).

II. EXPERIMENTAL

The structures of all the newly synthesised compounds, reported herein, were confirmed on the basis of their chemical properties, elemental analysis and spectral data. UV-Vis spectra were recorded in ethanol solvent. IR spectra were recorded on Perkin-Elmer spectrophotometer in the range 4000-400 cm^{-1} in KBr pellets. ^1H NMR spectra were recorded on Bruker Avance-II 400 NMR spectrophotometer in $CDCl_3$ using TMS as an internal standard. The melting points were recorded by capillary method in paraffin using Thiele's apparatus and all are uncorrected. Chemicals used were of A.R. Grade. The purity of newly synthesized compounds was checked by TLC using solvent combination.

Preparation of 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-N-[(2'-hydroxy-5'-chloro phenyl)ethanonylamino]-4,5-dihydro-Δ^2-pyrazoline (2):

1-Phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-amino-4,5-dihydro-Δ^2-pyrazoline (1) (0.01M) was refluxed with 1-(2-hydroxy-5-chlorophenyl)-2-bromoethanone (1a) (0.01M) in absolute ethanol for about 1 hour. After cooling, the reaction mixture was decomposed in ice-cold water. The product, thus separated, was filtered and crystallized from ethanol to get the compound 2.

M.F. $C_{30}H_{23}N_3O_4Cl_2$ (2): Brown crystalline solid, m.p.64 ^oC, yield 76 %, Elemental analysis (%): **C** 64.21/64.29; **H** 4.09/4.14; **N** 7.42/7.50; **O** 11.29/11.42; **Cl** 12.58/12.65. **UV** (ethanol): λ_{max} 670 nm, $n \rightarrow \pi^*$ transition. **IR** (KBr) (cm^{-1}): 3600-2400 (-OH stret.), 3085.52 (Ar. C-H stret.), 2918.54 (Ali. C-H stret.), 1650.16 (C=O stret.), 1566.35 (C=N stret.), 1354.30 (C-N stret.), 1209.32 (C-O stret.). 772.32 (C-Cl stret.). **^{1}H NMR** (δ ppm): 2.6 (s, 2H, -C**H**$_2$), 1.6 (s, 1H, -N**H**), 1.2 (d, 1H, C**H**-CH-CO-Ph), 1.61 (d, 1H, CH-C**H**-CO-Ph), 6.9-8.2 (m, 16H, Ar-**H**), 12.13 (s, 1H, H-bonded -O**H**).

Preparation of 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chlorophenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (3):

1-Phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-N-[(2'-hydroxy-5'-chloro-phenyl) ethanonylamino]-4,5-dihydro-Δ^2-pyrazoline (2) (0.01M) was refluxed with potassium thiocyanate (0.01M) for 4 hours in glacial acetic acid. After cooling, the reaction mixture was poured into ice-cold

water and the product, thus separated, was crystallized from ethanol-acetic acid mixture to get the compounds 3.

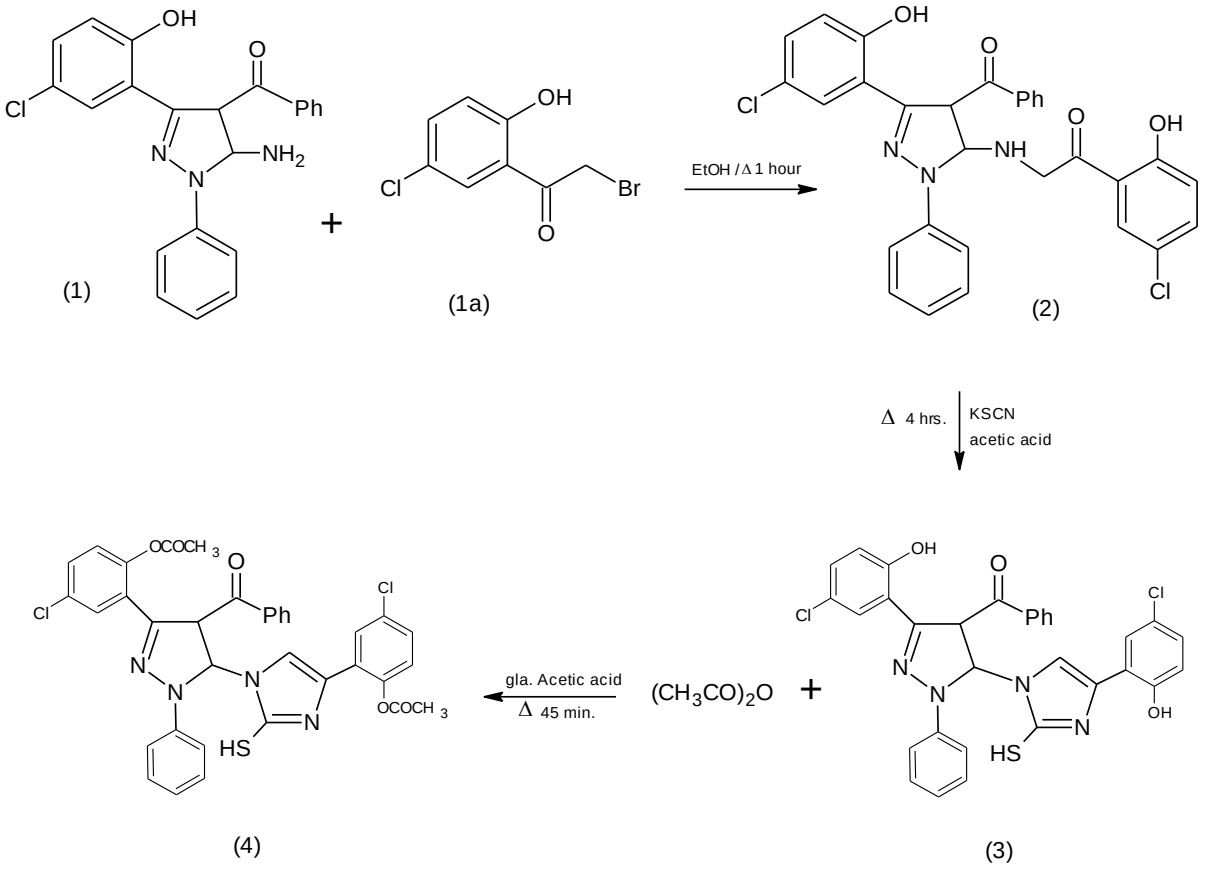
M.F. $C_{31}H_{22}N_4O_3SCl_2$ (3): brown crystalline shining solid, m.p.108 ^oC, yield 77 %, Elemental analysis (%): **C** 61.83/61.90; **H** 3.57/3.69, **N** 9.26/9.31, **O** 7.86/7.98, **S** 5.27/5.33, **Cl** 11.67/11.79. **UV** (ethanol): λ_{max} 540 nm, n→ π* transition. **IR** (KBr) (cm^{-1}): 3500-2400 (O-H stret.), 3084.45 (Aro.C-H stret.), 2916.44 (Ali. C-H stret.), 2532 (S-H stret.), 1647.26 (C=O stret.), 1633.33 (C=N stret.), 1600.33 (C=C stret.), 771.31 (C-Cl stret.).**^{1}H NMR** (δ ppm): 6.7 (s, 2H, C-**H**), 1.5 (d, 1H, C**H**-CH-CO-Ph), 2.5 (d, 1H, CH-C**H**-CO-Ph), 6.87 (s, 1H, N-C**H**=C), 7.1-8.1 (m, 16H, Ar-**H**), 7.86 (s, 1H, -C**H**=CH), 12.06 (s, 1H, O-**H**).

Preparation of 1-phenyl-3-(2-acetyloxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-acetyl-oxy-5'-chlorophenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (4):

1-Phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chloro phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (3) (0.01M) was refluxed with acetic anhydride for 45 min. in glacial acetic acid. After cooling, the reaction mixture was decomposed in water and the product, thus separated, was crystallized from ethanol-acetic acid mixture to get the compound 4.

M.F. $C_{35}H_{26}N_4O_5SCl_2$ (4): Brown solid, m.p. 89 ^oC, yield 69 %, Elemental analysis (%): **C** 61.25/61.32, **H** 3.74/3.82, **N** 8.13/8.17, **O** 11.53/11.67, **S** 4.64/4.68, **Cl** 10.28/10.34. **UV** (ethanol): λ_{max} 570 nm, n→ π* transition. **IR** (KBr) (cm^{-1}): 3084.39 (Ali. C-H stret.), 1640.25 (C=O stret.), 1645.78 (C=N stret.), 773.29 (C-Cl stret.). **^{1}H NMR** (δ ppm): 6.8 (s, 2H, C-**H**), 6.80 (d, 1H, C**H**-CH-CO-Ph), 6.86 (d, 1H, CH-C**H**-CO-Ph), 7.1-8.1 (m, 16H, Ar-**H**).

SCHEME:



III. ANTIMICROBIAL SCREENING

The compounds 1, 2, 3 and 4 were assayed against *Mushroom* crop pathogens using cup plate diffusion method. The inhibitory effects of compounds against these organisms are given in Table 1.The screening results indicate that the compound 1, 2, 3 and 4 showed good to moderate antifungal and antibacterial activities against fungi *Gliocladium roseum (Link) Bainier*, *Verticillium fungicola* and Bacteria *Pseudomonas stutzeri*, *Pseudomonas alcaligens, Pseudomonas fluorescene, Burkholderia gladioli.*

In this method, potato carrot agar and nutrient agar were melted, cooled and poured into sterile petri-plates and allowed for solidification. After solidification, by using Lawn method the fungal organisms were inoculated on the petri-plates having potato carrot agar and the bacterial organisms were inoculated on the petri-plates having nutrient agar. After some time, the cups of about 10 mm diameter were cut with the help of sterile borer. The drops of melted agar were added to seal the bottom of the cups and the wells were filled by pipetting 0.1 ml solution of test compounds 100 µg ml^{-1}.

The discs of *Cabendizum* (10mcg/disc) and *Gentamycine* (10mcg/disc), were used as positive controls. The zones of inhibitions were recorded in millimetres by using Himedia Zone Reader Scale.

the results obtained in the antimicrobial study are given in table no. 1

Table 1: Antimicrobial screening of titled compounds against Oyster mushroom crop pathogens.

S.N.	Compounds	Zone of inhibition (mm)					
		Fungal pathogens		Bacterial pathogens			
		Gliocladium roseum	*Verticillium fungicola*	*Pseudomonas stutzeri*	*Pseudomonas alcaligens*	*Pseudomonas fluorescene*	*Burkholderia gladioli*
1.	1	08	08	09	07	09	09
2.	2	06	07	05	06	08	04
3.	3	11	16	13	13	15	09
4.	4	09	04	07	08	11	07
5.	*Carbendizium*	09	09	NA	NA	NA	NA
6.	*Gentamycine*	NA	NA	08	08	08	08

IV. GROWTH PROMOTING IMPACT OF TEST COMPOUNDS

The spawns of experimental species *P. sajor-caju* ie *P. pulmonarius* were procured from genuine agricultural agencies and cultivated in the culture house of the ICAR affiliated Krushi Vidyan Kendra, Durgapur (Badnera) Dist. Amravati.

The experimental setup was divided into two parts ie 'A'-control group plants and 'B'-treated group plants. The spawns were inoculated and cultivated by the conventional methods.

The soyabean straw was used as a substrate for the cultivation of *Pleurotus sajor-caju* and it was firstly chopped into smaller pieces up to 3-5 cm and soaked in water tank for 12-15 hours. This was subjected for sterilization using hot water treatment maintained at 60-80 ^{o}C for 1 hour. The sterilised substrate was taken out and allowed to lower down the temperature.

The uniform size beds were prepared in sterilized polythene bags filled with alternate layers of sterilized soybean straw and spawns treated with the solution of test compounds. The mouth of packets (beds) were plugged and tightened with threads and 20-25 pin-holes were made on all sides of the packets. Similarly the untreated spawns were filled in control group beds (bags).

After proper labelling, the packets were hanged to iron racks and incubated in cultivation room on or below 25^{o}C. for mycelium running for 25-30 days. During this incubation period, appropriate temperature of the incubation room was maintained.

After the complete development of mycelium, the packets were taken out of the incubation room and shifted to growing room, where the packets were hanged to bamboo frame. During the harvesting of mushroom beds were irrigated according to need.

When the first primordial initiation was observed, the test compounds were sprayed on the mushroom with specific intervals. Mushroom crop was harvested before the fruiting body showed any splitting on the edges. The yields of mushroom crop from various bags with different parameters *viz* length, diameter, weight and colour were recorded.

The results of field experiments with test compounds are tabulated in table no. 2 and also shown in fig. no. 1 and 2:

Table 2: Effect of titled compounds on Oyster mushroom: Pleurotus sajor-caju spp.

Treated bags	Compo-unds	D (cm)	T (cm)	L (cm)	Weight of Dry Bags (gm) (After Harvesting)	Total Weight (gm)		Colour
						Fresh	Dry	
1.	1	8.0	0.5	5.8	0.930	219	20.45	White
2.	2	8.7	0.4	5.8	0.992	198	17.61	White
3.	3	11.7	0.6	6.9	0.983	227	21.35	Creamy
4.	4	11.6	0.5	6.3	0.955	207	18.93	Creamy
5.	1,4-Dioxane	6.0	0.4	6.1	0.990	176	19.13	White
6.	Control	6.8	0.3	5.5	0.853	204	20.00	White
D = Diameter ; T = Thickness ; L = Length								

V. ANALYSIS OF MUSHROOM SAMPLES TREATED WITH TEST COMPOUNDS

The samples of *P. sajor-caju* collected during the experimental study of growth promoting impact were sun-dried and immediately proceeded for analysis of % crude fibre, % crude protein and elemental detection with special reference to N, P, K and S.

The analysis of crude fibre percentage of the samples was carried out at Food Testing Laboratory, Krishi Vigyan Kendra, Durgapur (Badnera) Dist. Amravati using Pelicans FBS-06 (P) Laboratory Manuals & AOAC Method, whereas percentage of crude protein and element detection were determined at Analytical Lab, using Leaf method of analysis. The Kjeldahls method, UV spectrophotometer and Flame photometer were used for the analysis of N, P, K and S elements.

The results of analysis obtained for treated mushroom samples are tabulated in table no. 3:

Table 3: Analytical results of dry Oyster mushroom: P. sajor-caju spp. treated with titled compounds.

S.N.	Sample	% of Crude Fibre	% of Crude Protein	% N	% P	% K	% S
1.	1	8.00	15.15	2.425	0.3042	2.560	0.1267
2.	2	8.73	16.02	2.564	0.3115	2.937	0.1369
3.	3	10.05	20.30	3.248	0.3238	2.710	0.1325
4.	4	9.85	18.93	3.029	0.2980	2.444	0.1317
5.	1,4-Dioxane	8.06	13.29	2.127	0.275	2.346	0.1358
6.	Control	5.64	15.98	2.558	0.367	2.747	0.1412

VI. RESULTS AND DISCUSSION

In the present study newly synthesized 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-amino-4,5-dihydro-Δ^2-pyrazoline (1), 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-N-[(2'-hydroxy-5'-chlorophenyl)ethanonylamino]-4,5-dihydro-Δ^2-pyrazoline (2), 1-phenyl-3-(2-hydroxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-hydroxy-5'-chloro-phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (3) and 1-phenyl-3-(2-acetyloxy-5-chlorophenyl)-4-benzoyl-5-[2-mercapto-4-(2'-acetyloxy-5'-chloro-phenyl)imidazolo]-4,5-dihydro-Δ^2-pyrazoline (4) were screened for their antimicrobial activity against some *Mushroom* crop damaging pathogens includes fungi *viz. Gliocladium roseum (Link) Bainier*, *Verticillium fungicola* and bacteria *viz. Pseudomonas stutzeri*, *Pseudomonas alcaligens*, *Pseudomonas fluorescene*, *Burkholderia gladioli*. From the results, it has been observed that the titled compounds showed good to moderate amount of antibacterial activity.

Pleurotus sajor-caju, a species of *Oyster mushroom* was treated with test compounds to examine the efficacy of the newly synthesised compounds on the morphology of treated mushroom species with inclusion of analysis of treated samples.

When the treated and control species of mushroom were compared with reference to their morphological characters, it was interesting to note that the treated species exhibited significant growth in diameter and thickness of caps as well as lengthening of stipes. In addition to this, there was remarkable increase in the yields because of that healthy growth and disease free environment.

The analytical results obtained for all the treated mushroom samples clearly show the increase in the value of crude fibre percentage as well as the crude protein percentage. The presence of elements like N, P, K and S were also analysed in the treated mushroom samples. The more vigorous observations revealed that the mushroom crop treated with imidazole blends of azoles were found more effective in the enhancement of crude fibre percentage compared to other treated compounds.

However, further investigation and a systematic approach in the light of agricultural science would certainly prove to be a potential tool for the growth promoting and creating ecofriendly environment for mushroom cultivation.

VIII. CONCLUSION

On the basis of chemical analysis and spectral data, it is concluded that, the synthesis of titled compounds was achieved successfully. The antimicrobial screening of these compounds showed good to moderate antifungal and antibacterial activities. From the Table-1 it can be noticed that the imidazolo-pyrazoline have great potential towards mushroom pathogens.

The treated species ie. *P. sajor-caju* showed significant growth with respect to diameter, thickness and lengthening of stipe that reflects the curative and growth promoting properties of the titled compounds. Besides this, enhancement of the yields reveals the healthy growth due disease free environment.

The newly synthesised compounds also showed noticeable enhancement in the nutritive values ie increase in crude fibre percentage and crude protein percentage. In this regard, the imidazole blends of azoles were found more effective in the enhancement of nutritive value.

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