Research Journal of Chemical Sciences _____E-ISSN 2231-606X
Vol. **6(5),** 17-21, May **(2016)** *Res. J. Chem. Sci.*

Synthesis, Characterisation and Screening of newly synthesised analogues of Imidazolo-Thiazoles and their Impact on growth of *Oyster mushroom spp.* (*Pleurotus sajor-caju*)

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Available online at: www.isca.in, www.isca.me
Received 22nd March 2016, revised 20th April 2016, accepted 10th May 2016

Abstract

The majority of farmers and mushroom growers in tropical region of country (Central India) has been experimenting with Oyster mushroom cultivation and are very worried about the very high temperature and susceptibility of mushroom crops towards the pathogens responsible for common crop diseases. This is the main constraint in the large scale cultivation of edible mushrooms like Oysters in this part of the country. As a consequence, ultimate yield of Oyster mushroom in Central India is adversely affected. Whenever, the crops fall prey to diseases, farmers try to control them by spraying some fungicides on them but in many cases they do not succeed. Basic understanding about the disease is a prior strategy to manage them. To safeguard the crop by various preventive and controlling measures which were elaborated by several workers with some basic aspects based on their research activity. Literature survey reveals that, the utility of imidazole substituted azoles in the fields of agricultural science and medicinal chemistry is ever-increasing. Owing to their importance in the field of agriculture as plant protecting and growth regulating agents, we thought it worthwhile to study the efficacy of newly synthesised heterocycles viz. 2-phenylamino-4-benzoyl-5-(2-hydroxy-5-chlorophenyl)-1,3-thiazole, 2-N-phenyl-N-[(2-hydroxy-5-chloro-phenyl)ethanonylamino]-4-benzoyl-5-(2'-hydroxy-5'-chlorophenyl)-1,3-thiazole, 2-[2-phenylthio-4-(2-hydroxy-5-chlorophenyl)imidazolo]-4-benzoyl-5-(2'-hydroxy-5'-chlorophenyl)-1,3-thiazole and 2-[2-phenylthio-4-(2-acetyloxy-5-chloro-phenyl)imidazolo]-4-benzoyl-5-(2'-acetyloxy-5'-chlorophenyl)-1,3-thiazole in the light of their significance towards growth promoting and disease controlling impact on Oyster mushroom crop. The results obtained in the present study are very encouraging.

Keywords: Phenylthiazole, Ethanonylamino-1,3-thiazole, Imidazolo-1,3-thiazole, Acetyloxy-imidazolo-1,3-thiazole, Antimicrobial activity, *Pleurotus sajor-caju.*.

Introduction

The ring system of five membered heterocyclic compounds contain heteroatoms at 1,3-positions are designated as *1,3-azoles.* In such rings, when both positions are occupied by nitrogen, then they are referred as *imidazole* and in case, nitrogen and sulphur are present at 1,3-positions they are called as *thiazoles.*

Together with the other derivatives of 1,2 and 1,3-azoles, the thiazoles soon constituted an important part of heterocyclic chemistry. Some substituted thiazoles were reported to have antibacterial[1], anti-cancer[2], antimicrobial[3], antioxidant[4], antihelmintic, insecticidal, antitumor[5], anticonvulsant, antifungal[6], cytotoxic[7] and anti-inflammatory[8] activities.

In the tropical region of our country (Central India), the majority of farmers and mushroom growers have been engaged in the production of *Oyster mushroom* crop but the yield they have produced did not commensurate with their efforts and investment since mushroom crop easily fall prey to infections caused by crop pathogens *viz. Gliocladium roseum (Link) Bainier*, *Verticillium fungicola, Pseudomonas stutzeri*, *Pseudomonas alcaligens, Pseudomonas fluorescene, Burkholderia gladioli* and thus became a major problem in the cultivation.

Literature survey reveals that imidazole blended thiazoles have great importance in the field of agriculture. Most of the pesticides have imidazoles analogue of thiazole as an active ingredients in their composition.

Besides these imidazole blends of azoles there are some compounds such as methyl-1-(2-methylthioethyl-carbamoyl)benzimidazol-2-yl- carbamate, 2-(3,5-dimethylpyrazol-1-yl) -benzimidazole, {(*RS*)-2-[3-(4-chlorophenyl)propyl]-2,4,4-trimethyl-1,3-oxazolidin-3-yl}-(imidazol-1-yl)-methanone and 2-(1,3-thiazol-4-yl)-benzimidazole are reported as major constituents of many pesticides and plant growth regulatory agents[9-11].

Owing to the importance of azoles in the field of agricultural sector as plant protecting and growth regulating agents, we thought it worthwhile to synthesise and study the efficacy of some 1,3-thiazoles *viz*. 2-phenylamino-4-benzoyl-5-(2-hydroxy-5-chlorophenyl) -1,3-thiazole, 2-N-phenyl -N- [(2-hydroxy-5-chlorophenyl) ethanonylamino] -4-benzoyl-5- (2'-hydroxy-5'-chlorophenyl) -1,3-thiazole, 2-[2-phenylthio- 4-(2-hydroxy-5-chlorophenyl) -imidazolo] -4-benzoyl-5- (2'-hydroxy-5'-chlorophenyl) -1,3-thiazole and 2-[2-phenylthio-4-(2-acetyloxy-5-chloro-phenyl) imidazolo] -4-benzoyl-5- (2'-acetyloxy-5'-chlorophenyl) -1,3-thiazole with special reference to their use as growth promoting and disease controlling agents for *Oyster mushroom spp.* viz. *Pleurotus sajor-caju*.

Methodology

The structures of all the newly synthesised compounds were confirmed on the basis of their elemental analysis, chemical properties and spectral data. UV-Vis spectra were recorded in ethanol solvent. IR spectra were recorded in KBr pellets. ^{1}H NMR spectra were recorded 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. The purity of newly synthesized compounds was tested by TLC using different solvent combination.

Preparation of 1-(2-hydroxy-5-chlorophenyl)-2-bromo-3-phenylpropane-1,3-dione (2): 1-(2-Hydroxy-5-chlorophenyl)-3-phenylpropane-1,3-dione (1) (0.01M) was treated with bromine in glacial acetic acid reagent (6.4 ml). After complete addition of reagent, the reaction mixture was kept at room temperature for about 30 minutes. The solid product, thus separated, was filtered and washed with a little petroleum ether to get the compound (2). (m.p. 73^{o}C, yield: 78 %).

Molecular Formula $C_{15}H_{10}O_3ClBr$ (2): Yellowish brown amorphous solid, m.p. 73^{o}C, yield 78 %, Elemental analysis (%): **C** 50.91/50.95; **H** 2.82/2.85; **O** 13.55/13.57; **Cl** 09.98/10.03; **Br** 22.57/22.60. **UV** (ethanol): λ_{max} 520 nm, n→ π* transition. **IR** (KBr) (cm^{-1}): 3222.46 (-OH stret.), 3070.46 (Ar. C-H stret.), 1958.66 & 1906.65 (Overtone bands), 1701.30 (C=O stret.), 1626.19 (C=O stret.), 771.81 (C-Cl stret.), 698.00 (C-Br stret.). **^{1}H NMR** (δ ppm): 5.12 (s, 1H, -CO-C**H**Br-CO-), 7.0-8.2 (m, 8H, Ar-**H**), 11.04 (s, 1H, Ar-O**H**), 6.7 (s, 1H, -C**H=**C-OH), 4.4 & 4.5 (s, 1H, -CH**=**C-O**H)**

Preparation of 2-phenylamino-4-benzoyl-5-(2-hydroxy-5-chlorophenyl)-1,3-thiazole (3): A mixture of 1-(2-Hydroxy-5-chlorophenyl)-2-bromo-3-phenylpropane-1,3-dione (2) (0.01M) and phenylthiourea (0.01M) was dissolved in ethanol (25 ml). To this aqueous potassium hydroxide solution (0.02M) was added and refluxed for 2.5 hours. After cooling, it was diluted with water and acidified with conc. HCl. The product, thus separated, was filtered and crystallized from ethanol to get the compound 3 (mp. 68 ^{o}C, yield: 77 %).

Molecular Formula $C_{22}H_{15}N_2O_2SCl$ (3): Pale yellow crystalline shiny solid**,** m.p. 68^{o}C, yield 77 %, Elemental analysis (%): **C** 64.92/64.94; **H** 3.69/3.72; **N** 6.84/6.88; **O** 7.83/7.86; **Cl** 8.68/8.71; **S** 7.85/7.88. **UV** (ethanol): λ_{max} 495 nm, n→ π* transition. **IR** (KBr) (cm^{-1}): 3600-2400 (-OH stret.), 3421.55 (-NH stret.), 3175.53 (Ar. C-H stret.), 1668.54 (C=O stret.), 1605.49 (C=N stret.), 1200.49 (C-O stret.), 804.54 (C-Cl stret.), 685.51 (C-Cl stret.). **^{1}H NMR** (δ ppm): 2.18 (s, 2H, -N**H-**Ph), 6.2 (h, 1H, -O**H**), 6.9-8.3 (m, 13H, Ar-**H**).

Preparation of 2-N-phenyl-N-[(2-hydroxy-5-chlorophenyl) ethanonylamino]-4-benzoyl-5-(2'-hydroxy-5'-chlorophenyl)-1,3-thiazole (4): A mixture of 2-Phenylamino-4-benzoyl-5-(2-hydroxy-5-chlorophenyl)-1,3-thiazole (3) (0.01M) and 1-(2-hydroxy-5-chlorophenyl)-2-bromo-ethanone (2b) (0.01M) refluxed in ethanol for about 1 hour. After cooling, the reaction mixture was decomposed in ice-cold water. The product, thus separated, was filtered and crystallized in ethanol to yield the compound 4 (m.p.152 ^{o}C, yield: 68 %).

Molecular Formula $C_{30}H_{20}N_2O_4SCl_2$ (4): Yellow solid, m.p. 152^{o}C, yield 68 %, Elemental analysis (%): **C** 62.56/62.61; **H** 3.44/3.50; **N** 4.82/4.87; **O** 11.07/11.12; **Cl** 12.27/12.32; **S** 5.55/5.57. **UV** (ethanol): λ_{max} 370 nm, n→ π* transition. **IR** (KBr) (cm^{-1}): 3500-2600 (-OH stret.), 3085.21 (Ar. C-H stret.), 2915.21 & 2848.21 (Ali. C-H stret.), 1645.14 (C=O stret.), 1614.15 (C=N stret.), 1560.15 (C=C stret.), 823.13 & 673.14 (C-Cl stret.). **^{1}H NMR** (δ ppm): 1.2 (s, 2H, -C**H**$_2$), 6.82 (s, 1H, C**H=C-**OH), 7.2-8.1 (m, 11H, Ar-**H**).

Preparation of 2- [2-phenylthio -4- (2-hydroxy -5-chlorophenyl) imidazolo] -4-benzoyl-5- (2'-hydroxy-5'-chlorophenyl) -1,3-thiazole (5): 2-N-phenyl-N-[(2-hydroxy-5-chlorophenyl) ethanonylamino] -4-benzoyl-5- (2'-hydroxy-5'-chlorophenyl)-1,3-thiazole (4) (0.01M) dissolved in glacial acetic acid (20 ml) was refluxed with potassium thiocyanate (0.01M) for about 4 hours. After cooling, the reaction mixture was poured in ice-cold water. The product, thus separated, was filtered and crystallized from ethanol to get the compound 5 (m.p.168 ^{o}C, yield: 67 %).

Molecular Formula $C_{31}H_{19}N_3O_3S_2Cl_2$ (5): Yellow amorphous solid, m.p. 168 ^{o}C, yield 67 %, Elemental analysis (%): **C** 60.35/60.39; **H** 3.10/3.11; **N** 6.79/6.82; **O** 7.76/7.79; **S** 10.37/10.40; **Cl** 11.45/11.50. **UV** (ethanol): λ_{max} 345 nm, n→ π* transition. **IR** (KBr) (cm^{-1}): 3600-2800 (-OH stret.), 3085.68 (Ar. C-H stret.), 2916.69 (Ali. C-H stret.), 1650.49 (C=O stret.), 1600.59 (C=N stret.), 1566.61 (C=C stret.), 771.57 (C-Cl stret.), 682.61 (C-Cl stret.). **^{1}H NMR** (δ ppm): 6.8 (s, 1H, Ar-O**H**), 7.5-8.1 (m, 17H, Ar-**H**).

Preparation of 2-[2-phenylthio -4- (2-acetyloxy -5-chlorophenyl) imidazolo] -4-benzoyl-5- (2'-acetyloxy-5'-chlorophenyl)-1,3-thiazole (6): 2-[2-Phenylthio-4-(2-hydroxy-5-chlorophenyl) imidazolo] -4-benzoyl-5- (2'-hydroxy-5'-chlorophenyl) -1,3-thiazole (5) (0.01M) was refluxed with

acetic anhydride for about 45 min. in glacial acetic acid. After cooling, the reaction mixture was decomposed in water and product, thus separated, was filtered and crystallized from ethanol to get the compound 6 (m.p. 107 ^{o}C, yield: 80 %).

Molecular Formula $C_{35}H_{23}N_3O_5S_2Cl_2$ (6): Yellowish crystalline solid, m.p. 107 ^{o}C, yield 80 %, Elemental analysis (%): **C** 59.93/60.00; **H** 3.27/3.31; **N** 5.94/6.00; **O** 11.37/11.42; **S** 9.09/9.15; **Cl** 10.07/10.12. **UV** (ethanol): λ_{max} 530 nm, n→ π* transition. **IR** (KBr) (cm^{-1}): 3500-2800 (-OH stret.), 2974.37 (Ali. C-H stret.), 1653.25 (C=O stret.), 1645.78 (C=N stret.), 804.54 (C-Cl stret.). **^{1}H NMR** (δ ppm): 6.8 (s, 2H, C-**H**), 7.1-8.1 (m, 12H, Ar-**H**).

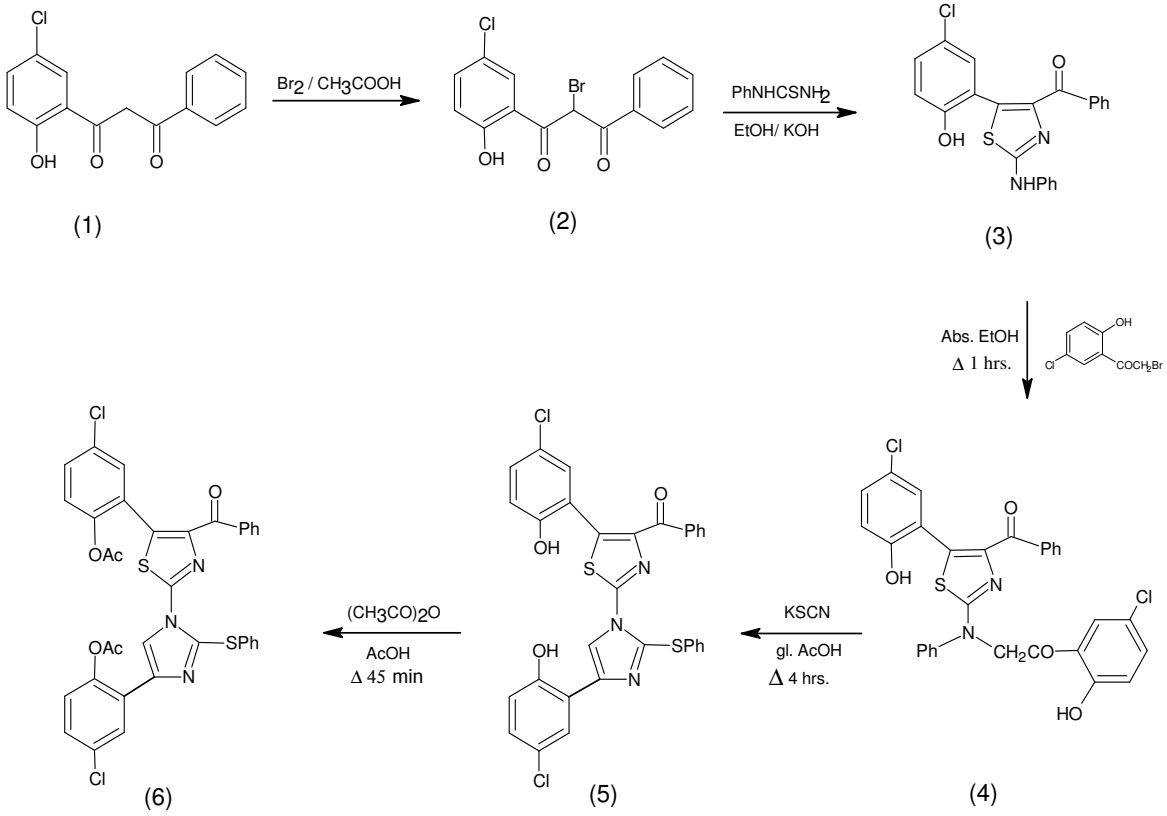
Antimicrobial Screening: The newly synthesised titled compounds 2, 3, 4, 5 and 6 were screened for their antifungal and antibacterial activity by cup plate method against causative organisms which are responsible for *Oyster mushroom* crop diseases *viz. Gliocladium roseum (Link) Bainier*, *Verticillium fungicola, Pseudomonas stutzeri*, *Pseudomonas alcaligens, Pseudomonas fluorescene* and *Burkholderia gladioli.* The inhibitory effects of compounds against these organisms are given in Table-1.

Impact of titled compounds on growth of Oyster mushroom: ***Pleurotus sajor-caju:*** The experiment was conducted at the ICAR affiliated Krushi Vidyan Kendra, Durgapur (Badnera) Dist. Amravati. A species of Oyster mushroom *Pleurotus sajor-caju* ie *P. pulmonarius* has been selected and cultivated in the specially made house.

The experimental setup was divided into two parts. **Part-A:** In this case the beds of control group crop spawns were inoculated and cultivated by the conventional method. **Part-B:** The beds of this group were treated with the solutions of test compounds.
Spawn treatment: The spawns of mushroom species were treated with the test compounds solution before inoculation in the respective beds.

Field treatment: The uniform size beds/packets of substrate (soybean straw) were prepared. The equal quantity spawns treated with the solutions of titled compounds were inoculated in the beds. The mouths of packets were tighten with threads and bags were incubated for mycelium growth on or below 25^{o}C.

After the completion of mycelium growth, the spawn packets were transferred to cultivation house and opened, also irrigated as per the need. When the first primordial initiated, the test compounds were sprayed with specific interval of time. 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, thickness, weight and colour were recorded (Table-2).



Scheme-1

Table-1
Antimicrobial screening of titled compounds against *Oyster mushroom* crop pathogens

Compounds	Zone of inhibition (mm)					
	Fungal pathogens		Bacterial pathogens			
	G. roseum	V. fungicola	P. stutzeri	P. alcaligens	P. fluorescene	B. gladioli
2	08	09	07	09	09	08
3	12	13	10	13	15	12
4	10	13	13.5	12	17	11
5	18	21	20.5	19	24	17
6	12	19	16.5	15	21	21
Carbendizium	09	09	NA	NA	NA	NA
Gentamycine	NA	NA	08	08	08	08

Table-2
Impact of titled compounds on growth of Oyster mushroom: *P. sajor-caju*

Treated bags	Compo-unds	D(cm)	T(cm)	L(cm)	Weight of Dry Bags (gm) (After Harvesting)	Total Weight (gm)		Colour
						Fresh	Dry	
1	2	8.1	0.4	6.0	0.992	189	18.20	Grey
2	3	9.5	0.6	6.4	0.867	228	22.95	Grey
3	4	8.8	0.5	6.2	0.923	210	19.88	White
4	5	11.4	0.6	7.0	0.988	239	24.37	Creamy
5	6	10.0	0.6	7.1	0.990	207	18.70	Grey
6	1,4-Dioxane	6.0	0.4	6.1	0.990	176	19.13	White
7	Control	6.8	0.3	5.5	0.853	204	20.00	White
D = Diameter ; T = Thickness ; L = Length								

Results and Discussion

In the present study the titled compounds 2, 3, 4, 5 and 6 were screened for their antimicrobial activity against some mushroom crop damaging pathogens. From the results it has been observed that the titled compounds showed good to moderate antifungal and antibacterial activity.

The impact of the titled compounds on the growth of *Pleurotus sajor-caju* was also studied. When the treated and control species of mushroom was compared with reference to their morphological characters, it was interesting to note that imidazole bends of azoles were found more effective in the enhancement of diameter and thickness of the cap as well as lengthening of stipe. However, the more vigorous observation reveals that the mushrooms treated with blends showed increase in the value of crude fibre percentage as compared to other treated compounds. As a consequence, there was increase in the yields.

Conclusion

On the basis of chemical analysis and spectral data, it is concluded that, the synthesis of titled compound were achieved successfully. The newly synthesised titled compounds are capable to cramp the growth of fungal and bacterial pathogens. The treated species shows significant growth in morphological characters that reflects the curative and growth promoting properties of the titled 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 an ecofriendly environment for mushroom cultivation in tropical belt of India.

Acknowledgement

We are thankful to CIL and SAIF, Panjab University, Chandigarh for providing spectral data and faculty of Department of Microbiology, SK College, Akola for antimicrobial screening. Also to Dr. K.A. Dhapke and Dr. A.N. Kakade, KVK, Durgapur (Badnera) Amravati for their consultancy in the oyster mushroom cultivation involved in the present study.

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