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Chemistry

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF PLANT PATHOGENS: A STUDY OF 4,5-DIHYDRO ISOXAZOLE DERIVATIVES

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ABSTRACT The series of novel substituted phenol derivatives containing isoxazole moieties 2-(5-(substituted)-4,5-dihydroisoxazol-3-yl)-4-methyl-6-nitrophenol and 2-(5-(substituted)-4,5-dihydroisoxazol-3-yl)-4-methylphenol have been designed and synthesized All the compounds were characterized via elemental analysis, IR, 1H, 13CMR and FAB-Mass analysis. The antibacterial and antifungal activities of were evaluated against two plant pathogenic bacteria and two plant pathogenic fungi.

KEYWORDS : Isoxazole, Plant Pathogens, Chalcones, Spectral Studies.

INTRODUCTON

Heterocyclic nitrogen and oxygen containing compounds have received considerable attention due to their significant bioactivities. During the last decades, intensive efforts have been undertaken to discover the highly active chemicals with favourable toxicological and environmental properties for the selective control of weed, insects and fungal disease. In several instances, dihydro isoxazole derivatives have been found as promising agrochemical products,,. The isoxazole constitute a fascinating class of five -membered heterocyclic compounds with one nitrogen and one oxygen ring atom. There are also some herbicidally, insecticidally or fungicidally active dihydro isoxazole derivatives known, which have been isolated from natural sources,. The dihydro isoxazole amino acids acivicin has been isolated from the fermentation broths of streptomyces sviceus and highly active against phytophthora infestans it is casual agnebts of potatolate blight, uncinula necator it is causal agent of grapes powdery mildew and the trehalase inhibiting dihydro isoxazole darevative trehazolin shows potent fungicidal activity against Rhizoctonia soloni it is causal agent of rice sheath blight".

Dihydro isoxazole have been widely applied as pharmaceuticals and agrochemicals because of their antifungal activities. Fluralaner, a commercial insecticide, is a successful example that introduces dihydro isoxazole as the scaffold,. Many studies have also focused on the antibacterial and antifungal activities of dihydro isoxazole derivatives,.

Concerning the synthesis of dihydro isoxazoles, the methods with stable and easily accessed starting materials and those conducted under mild conditions are more acceptable. Usually, the sophisticated way to synthesize dihydro isoxazole is by the cyclization of α, β-unsaturated carbonyl compounds (chalcones) with hydroxylamine. Chalcones can be easily obtained from a simple Claisen–Schmidt reaction,.

Keeping the above observations in view, we designed new compounds by introducing a 2-chloro benzaldehyde, 3-chloro benzaldehyde and indol-3-caoboxyaldehyde structure into a dihydro isoxazole scaffold, which will be expected to exhibit higher fungicidal bactericidal activities through the coexistence of two kinds of pharmacophores,,,.

MATERIALS AND METHODS

The chemicals and solvents used were of highest purity purchased commercially from Merck, S.D. Fine and Alfa Aesar Company Ltd. The melting points of all the synthesized compounds were recorded by Thiele's melting point apparatus as uncorrected values.

The elemental analysis was carried out on Thermo Scientific CHNS elemental analyser. IR spectra were recorded on a Shimadzu instrument using KBr pellets. ^{1}H NMR spectra were scanned by Brucker at 400 MHz using DMSO-d6 as solvent and TMS as an internal reference. ^{13}C NMR spectrum of a sample was recorded on same instrument at 100 MHz. Experimental procedure for synthesis of 2(5-(substituted phenyl)-4,5-dihydroisoxazol-3-yl)-4,6-substituted phenol (5a–5f).

Preparation of p-methylphenyl acetate (1)

The p-cresol was refluxed along with acetic anhydride and anhydrous sodium acetate for an hour. The reaction mixture was cooled and poured into the ice-cold water containing crushed ice. Acetate layer was separated by means of separating funnel and several times washed with water. It was finally purified by distillation and the distillate fraction was collected at about 236^{0}C, to get the compound (1) b.p. 236^{0}C yield: 84.74%.

Preparation of 2-hydroxy-5-methyl acetophenone (2)

p-methyl phenyl acetate (1) was mixed with anhydrous $AlCl_3$ (1) and heated at 120^{0}C for 45minutes on an oil bath. The reaction mixture was decomposed in ice cold water containing 10% hydrochloric acid and allowing the solution to fall drop by drop into ice cold water with constant stirring. Green solid compound i. e crude ketone (2) was obtained, m.p. 47^{0}C, yield: 89%.

Preparation of 2-hydroxy-3-nitro-5-methyl acetophenone (3):

2-hydroxy-5-methyl acetophenone (2) was dissolved in acetic anhydride in a beaker and reaction mixture was kept in ice bath by maintain temperature below 5^{0}C. To this reaction mixture conc. HNO_3 was added dropwise with constant stirring till the solution becomes orange coloured and kept for 4-5 hrs. it was then decomposed with ice cold water. Yellow granules obtained were filtered and washed with water and then crystallized from ethanol, m.p. yield: 72%.

Preparation of β-unsaturated chalcones (4a–4f)

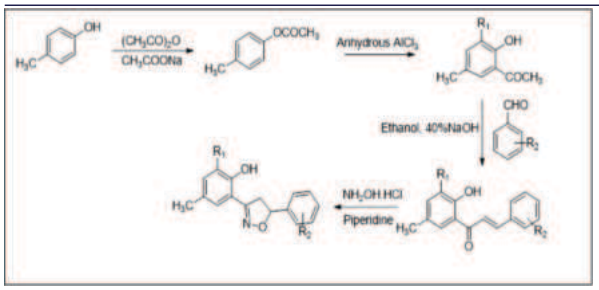
In this study, α, β-unsaturated chalcones 1 were synthesized by using a Claisen–Schmidt reaction,,,. To a solution of substituted acetophenone (0.01mol) and substituted aldehyde (0.01mol) in 15ml of ethanol and 40% sodium hydroxide solution added drop by drop.

The reaction mixture was continuously stirred on magnetic stirrer at room temperature, up to cake formation followed by decomposition with ice cold HCl (1:1). The crude Chalcones precipitate out ware filtered, washed with 10% NaHCO3 solution and then recrystallized from ethanol to obtained compounds (4a-f).

Preparation of 2(5-(substituted phenyl)-4,5-dihydroisoxazol-3-yl)-4,6-substituted phenol (5a–5f)

To a solution of chalcones (0.01 mol) and hydroxylamine hydrochloride (0.02mol) was reflux in 30 mL of ethanol and piperidine (0.5ml) for about 1.5 hrs. Subsequently, the reaction mixture was cooled and poured onto crushed ice acidified with dil. HCl, and then formed solids was collected and washed first with sodium bicarbonate solution (10%) and then with water to obtain crude products of 5a–5f.

Scheme of 2(5-(substituted phenyl)-4,5-dihydroisoxazol-3-yl)-4,6-substituted phenol (5a–5f).



R_1= H, NO_2; R_2=2-Cl, 3-Cl, Indol-3-carboxyaldehyde

Antibacterial and Antifungal activity assay

Tested strains (Pseudomonas, Xanthomonas, Cercospora and Cadophora) were obtained from the Institute of Plant Protection, Chandigarh. The bacterial and fungal strains were maintained in a potato dextrose agar medium (PDA) medium and stored at 4°C. Evaluation of the bactericidal and fungicidal activities was performed according to reported protocol inhibitory activities against two plant-pathogenic fungi was tested in vitro using the mycelia growth test on a PDA medium. Briefly, title compound solutions (in DMSO) and sterile molten PDA were mixed to obtain a final concentration of 100μg·mL^{-1} (containing 5% DMSO). Then the mixture was poured into 90 mm Petri dishes (15 mL·dish^{-1}), on which 10 mm mycelial disks of the two bacteria and two fungi were planted in the centre. Three replicates were conducted for each treatment. DMSO (5% contained in PDA) was used as a negative control, while thiabendazole and azoxystrobin were tested as positive controls. After a 24 hrs incubation period at 37°C (until the colonies in the control treatments had covered two-thirds of the Petri dishes), the mycelial growth diameters were measured using the cross-bracketing method, and the percentage of mycelial growth inhibition was then calculated. The inhibition percentages were calculated with the following equation: I=[(C−T)/(C−5)] ×100%, where T is the mycelial diameter (mm) in the Petri dishes with compounds and C is the diameter (mm) of the DMSO control. Furthermore, the antibacterial and antifungal activities of higher effective compounds (according to the preliminary assay) were evaluated using the inhibition percentages of tested compounds in the final series concentration. The EC50 values were calculated by linear-regression analysis.

RESULTS AND DISCUSSION

1. Chemistry

The reaction sequences for the synthesis of 2(5-(substituted phenyl)-4,5-dihydroisoxazol-3-yl)-4,6-substituted phenol (5a–5f) is outlined in above Scheme according to the reported method. The intermediate chalcones, (E)-3-(Substituted)-1-(2-hydroxy-5-methyl-3-substituted phenyl)prop-2-en-1-one, a kind of α,β-unsaturated ketones, were prepared by reacting the 2-chloro benzaldehyde, 3-chloro benzaldehyde and indol-3-carboxyaldehyde with substituted acetophenones via a facile procedure in the presence of a base with 60–85% yield. The chalcones were then converted into the 4,5-dihydro isoxazole derivatives through different reactions. The 4,5-dihydro isoxazole derivatives 5a–5f were prepared by refluxing a mixture of chalcones, hydroxylamine hydrochloride and piperidine in an ethanol medium with 67-85% yields. Compounds chalcones and isooxazolines were separated using column chromatography (ethyl acetate-petroleum ether). All title compounds were confirmed by IR, NMR and structural analysis data were shown below tables.

Table 1: Physical and analytical data of Compounds.

Compounds	Mol. Formula	Colour	M.P.	C%	H%	N%	Cl%
				Found/ Calculated			
5a	C16H14ClNO2	Pale yellow	101oC	66.77/66.79	4.60/4.90	4.80/4.87	12.00/12.32
5b	C16H14ClNO2	Pale yellow	1220C	66.77/66.79	4.60/4.90	4.80/4.87	12.00/12.32
5c	C18H16N2O2	Pale yellow	1320C	73.90/73.95	5.60/5.52	9.48/9.58	-
5d	C16H13ClN2O4	Dark red	1220C	57.73/57.76	3.91/3.94	8.39/8.42	10.62/10.65
5e	C16H13ClN2O4	Dark red	119oC	57.73/57.76	3.91/3.94	8.39/8.42	10.62/10.65
5f	C18H15N3O4.	Yellow	950C	64.04/64.09	4.42/4.48	12.46	-

5b: IR(KBr, cm-1): 3350(Hydrogen bonded -OH), 3743.83 (-C=N stretching), 3082.25 (-C-H stretch in aromatic), 2926.01 (-C-H aliphatic), 1577.77 (-C=N- stretching), 1083.99 (-C-O stretching), 906.54 (-N-O stretching isoxazole), 786.96 (-C-Cl stretching) ; 1-HNMR($CDCl_3$): δ 2.39 (s, 3H, CH_3), 2.47 (s, 3H, SCH_3).

5d: IR(KBr, cm-1): 3741.90 (Hydrogen bonded -OH), 3743.83 (-C=N stretching), 3068.75 (-C-H stretch in aromatic), 2926.01 (-C-H aliphatic), 1686.64 (-C=N- stretching), 1539.20 (-NO_2 stretching (asymmetric)), 1473.62 (-C=C- stretching), 1045.42 (-C-O stretching), 906.54 (-N-O stretching isoxazole), 752.24 (-C-Cl stretching) ; 1-HNMR(CDCl3): δ 8.0 (d,1H, Ar-H), 7.2 (d,1H, Ar-H), 4.89 (S, 1H, Phenolic -OH), 2.5 (s, 3H, -CH_3), 7.3-7.5 (m, 4H, Ar-H).

5f: IR(KBr, cm-1): 3741.90 (Hydrogen bonded -OH), 3743.83 (-C=N stretching), 3080.32 (-C-H stretch in aromatic), 2926.01 (-C-H aliphatic), 1686.64 (-C=N- stretching), 3221.12 (-N-H stretching in aromatic)1535.34 (-NO_2 stretching (asymmetric)), 1473.62 (-C=C-stretching),; 1-HNMR(CDCl3): δ 6.9 (d,1H, Ar-H), 7.2 (d,1H, Ar-H), 4.7(S, 1H, Phenolic -OH), 2.5 (s, 3H, -CH_3), 7.3-7.5 (m, 4H, Ar-Indol), 8.1 (d,1H,Ar-H), 7.8 (s,1H, N-H).

Antimicrobial Activity in vitro

As shown in table 2 given below , preliminary determination of the inhibition activities of title compounds (100 μg·mL^{-1}) against plant-pathogenic bacteria and fungi (Pseudomonas, Xanthomonas, Cercospora and Cadophora) suggested that all of the compounds showed significant antibacterial and antifungal activities against plant-pathogenic bacteria and fungi, with an inhibition rate higher than 50%. Among the tested bacteria and fungi, Pseudomonas and Cadophora ware completely inhibited by half of the tested compounds with an inhibition rate higher than 90%. It was obvious that these derivatives are particularly efficient against the mycelia growth of Pseudomonas and Cadophora in vitro.

Table 2. Antifungal activities of titled compounds at 100μg.mL^{-1} in vitro

Compounds	Bacteria		Fungi	
	Pseudomonas	Xanthomonas	Cercospora	Cadophora
5a	97.95	75.29	69.56	93.25
5b	96.01	69.27	56.28	94.34
5c	94.37	70.35	73.59	93.25
5d	93.25	55.15	78.29	91.15
5e	93.25	80.22	65.22	90.79
5f	93.25	62.79	57.54	93.25
Thiabendazole	91.20	60.23	65.45	90.32
Azoxystrobin	8765	72.36	57.61	89.54

Among newly synthesized compounds, 5a and 5b showed more potential bioactivities against Pseudomonas than those of thiabendazole and azoxystrobin. Meanwhile, 5a,5b,5c,5d,5e and 5f and showed higher fungicidal activities against cadophora. Surprisingly, the compounds containing a chlorine atom (5a,5b,5d and 5e) displayed excellent antifungal activities, even higher than commercial fungicides (thiabendazole and azoxystrobin). Therefore, the fungicidal activities of the title compounds closely depended on the core structure, substituent groups, and substituent positions Among the tested fungi, cadophora is a serious pathogenic fungus on gram and other cereals, and Cercospora is a harmful fungus on soyabean, various vegetables and crops worldwide. All the tested novel compounds have strongly effective for bacteria Xanthomonas. The title compounds showed significantly antifungal activities against these notorious fungi, suggesting that novel compounds have potential and should undergo further evaluation in vivo or in the field. In the present study, the 4,5-dihydro isoxazole derivatives showed significant antibacterial and antifungal activities against the tested bacteria and fungi, and approximately half of the title compounds exhibited higher inhibitory activities.

CONCLUSIONS

The series of novel 4,5-dihydro isoxazole compounds derived from p-cresol were designed and synthesized and were characterized via 1 H-NMR, 13C-NMR, and HRMS. Results of the preliminary biological activity assay indicated that most of the title compounds showed potential antibacterial and antifungal activities against Pseudomonas, Xanthomonas, Cercospora and Cadophora. Compound 5a and 5b, displaying significant bactericidal and fungicidal activities against

bacteria Pseudomonas and fungus Cadophora are worth being further evaluated in vivo and in the field. Further optimizations of 4,5 dihydro isoxazole derivatives should be carried out to develop more effective antibacterial and antifungal activities.

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