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Title Key: Biotransformation of chalcone by *pseudomonas sp.*

Biotransformation of chalcone by *pseudomonas sp.* Isolated from waste water of aurangabad

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Abstract

Chalcones are the precursors of flavonoids biosynthesis used as a starting material for synthesis of various heterocyclic compounds. *Pseudomonas* sp are capable of converting chalcones to isoxazilone by cyclization of alpha, beta position in the presence of hydroxylamine hydrochloride at 30 ^{0}C temperature in Minimal salt medium. . Locally isolated *Pseudomonas* from waste water of Aurangabad used to convert pyrazole chalcones to isoxazilone on flask scale using MSM, supplemented with yeast extract, glucose, peptone in different concentrations under controlled conditions. Biotransformation of pyrazole chalcone to isoxazilone was confirmed by H1NMR, IR,Mass spectral analysis.

Keywords: Chalcone, biotransformation, cyclization, *Pseudomonas.*

1. Introduction

Chalcones are also called as α, β-unsaturated ketones. They are precursors of flavonid biosynthesis. They can be obtained from natural sources or by synthesis. The presence of (functional group) in chalcones confers biological properties, i.e. bacteriostatic/ bactericidal activity. Chalcones are of a high interest due to their use as starting materials in the synthesis of a series of various heterocyclic compounds and especially chalcones bearing oxygenated function on the aromatic rings are the precursors of all flavonoids. Thus the synthesis of chalcones has generated vast interest to organic as well as for medicinal chemist. This class exhibits a broad spectrum of biological properties including, anti-inflammatory, antitumor, antibacterial, antifungal and antileischmanial. Some chalcones derivatives also showed a profound influence on the cardiovascular, cerebrovascular and neuromuscular systems including the vital organs of the experimental animals. Newly synthesized chalcones (1,3-diarylpropen-1-ones) and their analogs as potential therapeutic agents for diseases of the cardiovascular system. Some new chalcones reported on CYPIA inhibitory action. Some newly

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synthesized 4-(hydroxy) substituted chalcones reported as antiproliferative agents.

On other side Pyrazole ring has wide applications in medicinal chemistry. It is also reported that, pyrazole derivatives are gained synthetic interest in recent years due to their broad spectrum of biological properties like anti-inflammatory, analgesic, antibacterial, and antifungal activities. In the present study, we have synthesized a series of similar active chalcones containing Pyrazole nucleus.

Biotransformation is a combinational work of analytical chemistry and microbiology. In biotechnology biotransformation stands for conversion of natural or synthetic precursors into products of increased value. The main reactions during microbial biotransformation are hydroxylation, dehydroxylation,O-methylation,O-methylation,glycosylation,dehydrogenation, hydrogenation, C ring cleavage of the benzo-γ-pyrone system, cyclization, and carbonyl reduction. Chalcones were regioselectively cyclized to flavanones. Hydrogenation of flavonoids was only reported on transformation of chalcones to dihydrochalcones.

Whole cell or enzymes used under many different conditions(ie.Free immobilized or two phase system).*Pseudomonas* species are Gram negative motile rods belonging to the family *Pseudomonaceae* and found in various environment. Their ability to utilize different organic compound as carbon and energy source as well as survival in the absence of nutrients has been attributed to their genetic versatility which translates into enhanced metabolic activity.

2. Materials and methods

All the chemicals used in the research work were of AR grade. Nutrient broth of Hi media along with other chemicals purchased from local chemical distributor Lab trading. The Pyrazole chalcone was obtained from department of chemistry.*Pseudomonas* species isolated from waste water of Aurangabad was used to inoculate in 50 ml of nutrient broth medium and shaken at 30^0C on 180 rpm for 24 hours. A 10% aliquot of culture ($5.65x10^7$ CFU/ml) was used to inoculate in 100 ml minimal salt medium (MSM) supplemented with 0.2 % yeast extract, 0.5 % glucose and 0.2 % peptone and 0.1% hydroxylamine hydrochloride. The culture was incubated at 30^0C on orbital shaker incubator at 180 rpm until OD_{600} was approximately 2.0 and then 200 mg of Pyrazole chalcone was inoculated.

The MSM containing Pyrazole chalcone was further incubated for 6 days and harvested by sterile centrifugation technique (8000 rpm for 20 min.). The sample was extracted by the same value of ethyl acetate three times. Organic fraction was collected dried over a bed of anhydrous sodium sulphate and solvent evaporated to dryness at 37^0C to remove ethyl acetate. The residues were dissolved in methanol.

Analysis of metabolites

TLC was carried out for six days by using solvent system ethyl acetate:petether (70:30vol/vol) and developed chromatograms were visualized by exposure to iodine vapors. Biotransformed metabolites were further analyzed by H1 NMR, Mass spectra and IR spectra.

Spectral data of pyrazole chalcone

^{1}H NMR spectra: ^{1}H NMR spectra of compounds were studied in DMSO-d_6 showed characteristic doublet signals near δ-7.40 and 7.80 due to olefinic α,β-

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unsaturated protons respectively. However, these doublets are coalesced (mixed) with aromatic protons. Phenolic protons (2'-OH) appeared as singlet near at δ 11.00 – 12. 00 was observed and aromatic protons as multiple around δ 7.00–8.00. Compounds containing aromatic methyl group the singlet near at δ 1.3-1.9 was observed. The ethylenic protons shift at downfield in aromatic region is the characteristic of system. These findings are in agreements with those observed by different workers[79, 80.]

^{1}H NMR (DMSO-d_6) : δ 1.3 (s, 3H, -CH_3), 7.0-8.0 (m, 10H, Ar-H + CH=CH), 11.8 (S, 1H, -OHppm.M.S. (m/z) 372 [M^+ ion], 337 [100], 218 [18], 155 [20], 140 [16], [% rel. intensity] 118 [30], 99 [15], 77[85]

IR spectra: IR spectra of chalcones showed characteristic band at near region 1640–1680 cm^1 due to >C=O stretching vibrations. Lowering of normal >C=O to the lower wave number is attributed to the presence of α,β-unsaturated and phenolic hydroxyl group at *ortho* position. All the chalcones showed absorption in the region 1590 –1630 cm^{-1} due to (-CH=CH-) ethylene double bond. A broad peak in between 3000 – 3400 cm^{-1} was observed due to phenolic – OH group. Beside these bands 680 – 800 cm^{-1} due to C-Cl stretching and 600 – 700 cm^{-1} due to C-Br appear when ever present in the respective compound. These assignments are in agreement with those observed by several research groups.[83, 84]

Mass spectra: The mass spectra of corresponding chalcones show their molecular formula weight. It is found to be in agreement with the literature.

3.Results and discussion

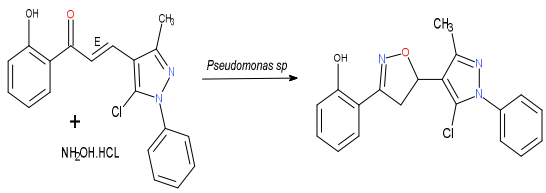
TLC of the broth reveals two different spots which were obtained by column chromatography and further analyzed by IR, H1NMR.

The IR spectra of compounds were recorded (in KBr pallets) on SHIMADZU spectrophotometer. ^{1}H NMR were recorded (in DMSO-d_6) on Avance-300 MHz spectrometer using TMS as an internal standard (chemical shifts are given in δ ppm). The mass spectra recorded on EI-SHIMADZU-GC-MS spectrometer.

IR spectra: The IR spectra showed characteristic absorption band at 1590-1598 cm^{-1} due to C=N stretching. A broad peak showed in between 3340-3374 due to the phenolic hydroxyl group.

H^1 NMR spectra: ^{1}H NMR of the isoxazoline showed following type of peak which confirmed the formation of product 3.42-3.81 (d, 2H, CH_2); 4.12-4.41, (m, 1H, CH) and 7.0-8.10 (m, Ar-H) ppm, 11-13 (s, 1H, OH) ppm. Besides these characteristic peak proton observed at expected region. These findings are in agreement with earlier researchers.[50]

Mass spectra: Mass spectra of the representative compound confirmed the molecular formula weight of that compound.These spectral analysis reveals that Pseudomonas sp convert pyrazole chalcone into isoxazoline by cyclization of alpha, beta position in the presence of hydroxylamine hydrochloride at 30 0C temperature in Minimal salt medium.



Pyrazole chalcone + Isoxazoline

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Hydroxylamine. HCL

The biotransformed product isoxazoline and its derivatives are useful as intermediates in the organic synthesis, polymers, and pharmacologically active materials. They possessing fungicidal, antimicrobial, bactericidal and mutagenic activities.Isoxazoline possess various biological and pharmacological activities.

4. Conclusion

Bacterial biotransformation is a new approach to modify thestructures of bioactive natural and synthetic flavonoids. Microbial factoriesshow advantages, for instance growing rapidly, ease of large-scaleproduction, environment-friendly, solvent-free, and so on. Furthermore, bacterial biotransformation improves the selectivity of naturalproducts without any toxic chemicals. The application of microbesfor biotransformation of chalcones led to form many novel flavonoidsin mixture by means of cyclization, hydroxylation, reduction, Odemethylation,and dehydrogenation reactions. In this research work pyrazole chalcone biotransformed to isoxazoline by *Pseudomonas* sp. isolated from waste water of Aurangabad. *Pseudomonas* capable of conversion of several natural and synthetic heterocyclic compound to other useful metabolites.

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