International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 169-172
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Biotransformation of Pyrazole Chalcones By *Conidiobolous Species* Isolated from Detritus Vegetable Waste

Nisar Ahmed* and Shaikh Abdul Baseer

Department of Microbiology, Sir Sayyed College Aurangabad.
Department of Chemistry, Sir Sayyed College Aurangabad.
Post Box no 89, Roshan Gate Aurangabad 431001(Maharashtra).

Received: 14 Jan 2019 / Accepted: 12 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: nisarmicro46@gmail.com

Abstract

Microorganisms showed their potential ability to degrade bioactive natural and synthetic compounds. *Conidiobolus* Sp. isolated from detritus vegetable waste was used for biotransformation of pyrazole chalcones under laboratory conditions on MSM (minimal salt medium) containing malt extract-0.1%, yeast extract-0.2%, glucose-0.5%, peptone-0.2% medium at 30°C for 10 days. After incubation sample was harvested and analysed by TLC, ^{1}H NMR, LR MS. The product which formed in the bio reduction reaction using *Conidiobolus species* was confirmed from spectral analysis was 1-(5-chloro-2-methyl-phenyl)-3-(5-chloro-3-methyl-1-phenyl-4H-pyrazol-4-yl) propan-1-one (ie reduced pyrazole chalcones). The biotransformation of pyrazole chalcone by *Conidiobolus* sp. reaction was regioselective, showing alternation of C-C double bond of the olefin and do not alter the carbonyl double bond of α, β unsaturated ketone system.

Keywords
biotransformation, *Conidiobolus* sp, pyrazole chalcones.

*****

INTRODUCTION

Microorganisms and their enzymes have proved to be versatile biocatalyst and are involved in the conversion of complex organic materials [1]. Microorganisms performed several chemical reactions as an alternative to obtain products of chemical and biological interest [2]. Biotransformation is a conversion of natural or synthetic precursors into products of increased value. Whole cell or catalytic enzyme used many different conditions such as free, immobilized. Biotransformation is a combinational work of chemistry and microbiology [3]. Such reactions certain advantages over the conventional reactions as performed in aqueous systems and at neutral pH, preventing the hazards of solvents in conventional synthesis [4-7].

Chalcones are α, β unsaturated ketones and precursors of flavonoids in which olefinic and carbonyl fragments are linked to an aromatic ring [6]. Chalcones can be obtained from natural sources or by synthesis. The functional group present on chalcone shows biological activity like antiviral activity14, inhibition of NS3 protease of dengue virus

DOI: https://doi.org/10.21276/ijpbs.2019.9.2.23

[9], activity against herpes simplex virus, HIV-1 replication inhibition in lymphocytes [10]. The main reaction during biotransformation are hydrogenation, dehydrogenation, O-methylation, glycosylation, hydroxylations, dehydroxylations, C-ring cleavage, cyclization and carbonyl reduction. Chalcones were regioselectively cyclized to flavones. Hydrogenation of flavonoids was reported on transformation of chlacones to dihydrocalcones [6].

The fungi *conidiobolus* are inhabitant of soil debris and generally found in India, America, Africa etc. [11] *Conidiobolus* shoot their spores and its mature spores are violently discharged and dispersed at some interval and germinate to form new colonies. *Conidiobolus* species also reported as occasional pathogens [11]. Biodegradation of chlorinated pesticide γ-hexachlorocyclohexane (lindane) by *Conidiobolus* 03-1-56 isolated from litter was reported which completely degraded lindane on the 5th day of incubation in the culture medium and GC-ECD studies confirmed that lindane removal did not occur *via* adsorption on the fungal biomass [12].

In present research work *Conidiobolus* species isolated from vegetable waste was used for the biotransformation of pyrazole chalcones.

MATERIALS AND METHODS

All the chemicals used in present research work were of AR grade. Melting points of samples were corrected and determined in an open capillary tube. IR spectra were recorded on FTIR Shimadzu spectrometer. ^{1}H NMR spectra were recorded in DMSO-d_6 on Advance 300 MHz spectrometer using TMS as an internal standard. The mass spectra were recorded on EI-Shimadzu-GC-MS spectrometer. Elemental analyses were performed on a Carlo Erba 106 Perkin-Elmer model 240 analyzer. Chalcones were synthesized in chemistry laboratory and used for biotransformation.

Synthesis of chalcones

General procedure for synthesis of chalcone derivatives an equimolar mixture of 5-chloro-3-methyl-1-phenyl-1Hpyrazole-4-carboxyaldehyde 2 (1 mmol), substituted acetophenone (1 mmol), KOH (2 mmol) were stirred in ethanol at 40 °C for 1 hr. After completion of the reaction (checked by TLC), the crude mixture was worked up in ice cold water (100 mL) and acidified with dil HCl. Solid get separated was filtered and dried. The crude product was crystallized from acetic acid. Similarly, other analogues of the series were synthesized by the same procedure [15].

IR spectra of chalcone showed the characteristic band at 1640-1650 cm-1 due to carbonyl stretching vibration. 1H NMR spectra of the compounds showed the aromatic protons**.** while the other aromatic and aliphatic protons were appeared at expected region. The mass spectra of the compounds showed molecular ion peak were correlated with their molecular weight of that respected compound.

Biotransformation of chalcones

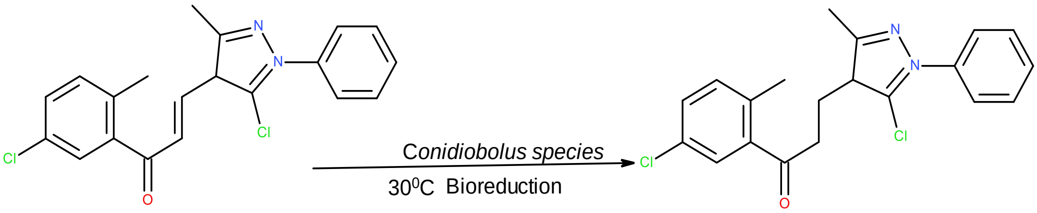
Conidiobolus isolated from vegetable waste was enriched on MGYP (malt extract-0.3 %, yeast extract-0.3%, peptone- 0.5%, glucose- 1%) and incubated at 30^{0}C for 48 hours. After enrichment the 2 ml of inoculum is transferred to 100 ml of MSM (minimal salt medium) containing malt extract-0.1%, yeast extract-0.2%, glucose-0.5%, peptone-0.2%. chemically synthesized chalcone 0.05 gm. dissolved in 0.5 ml DMSO was added under sterile conditions in 250 ml of Erlenmayer flask and shaken at 160 rpm at 30°C. After 10 days the sample was harvested by sterile centrifugation technique (8000 rpm for 20 min.) and extracted by the same value of ethyl acetate three times.

The organic phase was grouped, dried using sodium sulphate (Na_2So_4) filtered and evaporated at reduced pressure. Experiments were carried out in triplicates and analysed by TLC. From one of the experiment purification was carried out. The crude residue 90 mg obtained from biotransformation of chalcone was chromatographed on silica gel with ethyl acetate 200 ml and methanol 100 ml. The ethyl acetate extract of 45 mg by TLC analysis indicate interested substance. This was further chromatographed sequentially on silica gel eluting with hexanes and increasing polarity of ethyl acetate and 11mg of fraction were obtained.

RESULT AND DISCUSSIONS

Chalcones (71% yield) was synthesized by the reported method. [15] and structure was determined by the analysis of 1D- and 2D-NMR, mass spectra and comparison with reported physical spectroscopic data. *Conidiobolus* species was used to biotransformed the obtained chalcones. Reduced chalcone was identified as a dihydrochalcone with 37% yield and identified as a new compound. Biotransfomation of chalcones showing alternation in C-C double bond of the olefin with no reaction at carbonyl double bond. Out of the different reactions reported none of the reaction showed regioselectivity for double bond C-2 and C-3. Shindo, Kagiyama, et al reported the unsubstituted chalcone was converted to 2”-hydroxychalcone and 2”, 3” –dihydroxychalcone in 25% and 59 % yield by *E. coli* [13]. Similarly, Herath W. et al reported biotransformation of chalcone xanthohumol using the culture broth of *Pichia membranifaciens* synthesized three metabolites, one isomeric prenylflavanone 3.3% yield and to modified chalcones in 0.55 and 0.58% yields [14].

Spectroscopic data of Reductive pyrazole chalcone IR (KBr): 1650 (>C=O), 1595 (-C=N); 1H NMR (DMSO-d6): 2.32 (s, 6H, CH3), 7.05-8.42 (m, 10H, Ar-H), 3.01(2H, t,J 7.5 Hz)beta and alpha 3.10 (2H, t,J 7.5 Hz) ppm; M.S. (m/z): 339[M+]; 341,343 Anal. Calcd for $C_{20}H_{20}ON_2Cl_2$: C, 70.02; H, 5.01; N, 7.5%. Found: C, 64.00; H, 5,01; N, 7.5%.



Pyrazole chalcone Reductive pyrazole chalcone

The product which formed in the bio reduction reaction using *Conidiobolus species* was confirmed from spectral analysis 1-(5-chloro-2-methyl-phenyl)-3-(5-chloro-3-methyl-1-phenyl-4H-pyrazol-4-yl) propan-1-one in NMR spectra that indicate reduction of double bond of the conjugated olefinic system and by the presence of two triplets. NMR spectra reveals signal due to substituted methylene proton observed at 3.01(2H, t J 7.5 Hz) β and α 3.10(2H, t J 7.5 Hz) this coupling of proton conjugated by HCOSY spectrum. From C13 NMR spectra carbonyl carbon increase about 10 ppm due to loss of conjugation. Analysis from LR Spectrum molecular ion pear 374.28 and presence of two chlorine observed along with fragmentation $C_{20}H_{19}Cl_2N_2O$.

CONCLUSION

Biotransformation is an effective tool for structural modification of biologically active natural and synthetic compounds such as chalcones. In this present study the pyrazole chalcones converted into reduced pyrazole chalcone with better yield. The biotransformation reaction was regioselective, showing alternation of C-C double bond of the olefin and do not alter the carbonyl double bond of α, β unsaturated ketone system.

ACKNOWLEDGEMENT

The Authors would like to thank Principal Dr. Shaikh Kabeer Ahmed, Sir Sayyed College for providing necessary instrumentation facility.

REFERENCES

[1] G. Shyam Prasad; Studies on microbial transformation of albendazole by soil fungi, Indian Journal of Biotechnology, Vol-8: 425-429, (2009)

[2] Marivaldo J. C. Correa, Fatema M Nunes etal; Biotransformation of chalcones by Endophytic fungus Aspergillus Flavus isolated from Paspalum maaritimum Trin, J of Braz. Chem. Soc, Vol 22(7): 1333-1338, (2011)

[3] Nisar Ahmed, Shikh Abdul Baseer; Biotransformation of Curcumin to vanillin by Pseudomonas species isolated from waste water of Aurangabad, J of Microbio and Biotech Res, Vol 5 (1): 8-10, (2015)

[4] Davis, B. G Boyer; Biocatalyst and enzymes in organic chemistry, Nat. Prod. Rep. Vol 18, 618, (2001)

[5] Comasseto J. V. Omori, A T Porto; Preparation of chiral organochalcogeno-α-methylbenzyl alcohols via biocatalysis. The role of *Daucus carota* root, Tetrahedron Lett. Vol 45, 473, (2004)

[6] Comasseto, Leandro Helgueira Andrade, Álvaro Takeo Omori; Deracemization of aryl ethanols and reduction of acetophenones by whole fungal cells of *Aspergillus terreus* CCT 4083, *A. terreus* CCT 3320 and *Rhizopus oryzae* CCT 4964, J of Mol. Catal B: Enzyme, Vol 29 55-61,(2004)

[7] Vicente Gotor-Fernández, Rosario Brieva, Vicente Gotor; Lipases: Useful biocatalysts for the preparation of pharmaceuticals, J of Mol. Catal B: Enzyme,Vol 40, 111-120, (2004)

[8] H Ishitsuka, Y T Ninomiya, C Ohsawa, M Fujiu, Y Suhara; Direct and specific inactivation of rhinovirus by chalcone Ro 09-0410. Antimicrob. Agents. Chemother, Vol 22, 617, (1982)

[9] Tan Siew Kiat, Richard Pippen, Rohana Yusof, Halijah Ibrahim; Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, *Boesenbergia rotunda* (L.), towards dengue-2 virus NS3 protease, Bioorg Med Chem Lett. Vol 16 (12), 3337-3340, (2006)

[10] Jiu-Hong Wu, Xi-Hong Wang, Yang-Hua Yi, Kuo-Hsiung Lee; Anti-AIDS agents 54. A potent anti-HIV chalcone and flavonoids from genus *Desmos*, Bioorg Med. Chem Lett, Vol 13(10), 1813-1815, (2003)

[11] Nisar Ahmed; Isolation of Conidiobolus Sp. from detritus vegetable waste, Vol 2(3), 262-265, (2017)

[12] Varima Nagpal, M. C. Srinivasan, M. Paknikar; Biodegradation of γ-exachlorocyclohexane (Lindane) by a non-white rot fungus conidiobolus 03-1-56 isolated from litter, Vol 48 (01), 134, (2008)

[13] Shindo, Kagiyama, Nakamura R, Hara A; Enzymatic synthesis of novel antioxidant flavonoids by *Escherichia coli* cells expressing modified metabolic genes involved in biphenyl catabolism,Vol 23(1), (2003)

[14] Herath W H M, Ferriera D, Khan I A; Microbial transformation of xanthomol, Vol 62(5), 673-677, (2003)

[15] Dawane, B. S.; Konda, S. G.; Shaikh, B. M.; Mandawad, G. G. Synthesis of Some Novel Substituted Pyrazole Based Chalcones and Their In-Vitro Antimicrobial Activity Asian J. Res. Chem. Vol 13(1), 90, (2010)

[16] Bhaskar S. Dawane. Shankaraiah G. Konda, Gajanan G. Mandawad, Baseer M. Shaikh; Poly (ethylene glycol) (PEG-400) as an alternative reaction solvent for the synthesis of some new 1-(4-(40-chlorophenyl)-2-thiazolyl)3-aryl-5-(2-butyl-4-chloro-1H-imidazol-5yl)-2-pyrazolines and them in vitro antimicrobial evaluation European Journal of Medicinal Chemistry, Vol 45 387–392, (2010)