JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Biotransformation of pyrazole containing substituted hydroxy chalcones by Basidiobolusfungus

Shaikh Abdul Baseer[1]*, Syed Nisar Ahmed[2]

[1]Department of Chemistry, Sir Sayyed College, Aurangabad.

[2]Department of Microbiology, Sir Sayyed College, Aurangabad.

Post Box No. 89, Roshan Gate, Aurangabad-431001(Maharashtra).

Abstract

Microorganisms showed their potential ability to degrade bioactive natural and synthetic compounds. *Basidiobolus* Sp. isolated from detritus vegetable waste was used for biotransformation of substituted hydroxy 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 30ºC for 10 days. After incubation sample was harvested and analyzed by TLC, ^{1}H NMR, LR MS. The product which formed in the reaction using *Basidiobolus species* was confirmed from spectral analysis was 1, 5-benzodiazepines.The biotransformation of pyrazole containing substituted hydroxy chalcones by *Basidiobolus* reaction was regioselective. 1, 5-benzodiazepines was described here by the condensation of chalcones with *o*-phenylenediamine.

Keywords: Biotransformation, *Basidiobolus* sp , chalcones, benzodiazepines.

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 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 [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 *Basidiobolus* are inhabitant of soil debris and generally found in India, America, Africa etc. [11] *Basidiobolus* shoot their spores and its mature spores are violently discharged and dispersed at some interval and germinate to form new colonies. *Basidiobolus* species also reported as occasional pathogens[11]. Biodegradation of chlorinated pesticide γ-hexachlorocyclohexane (lindane) by *Basidiobolus* 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 *Basidiobolus* species isolated from vegetable waste was used for the biotransformation of pyrazole containing substituted hydroxy 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. Pyrazole containing substituted hydroxy chalcones were synthesized in chemistry laboratory and used for biotransformation.

Synthesis of pyrazole containing substituted hydroxy chalcones

General procedure for synthesis of pyrazole containing substituted hydroxy chalcones derivatives contain an equimolar mixture of 5-chloro-3-methyl-1-phenyl-1Hpyrazole-4-carboxyaldehyde 2 (1mmol), 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 pyrazole containing substituted hydroxy chalcones

*Basidiobolus*isolated from vegetable waste was enriched on MGYP(malt extract-0.3 %, yeast extract-0.3%, peptone- 0.5%, glucose- 1%) and incubated at 30^0C 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 were 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. *Basidiobolus* species was used to biotransformed the obtained substituted hydroxy chalcones. Biotransfomation of pyrazole containing substituted hydroxy chalcones showing condensation reaction by *Basidiobolus* as metabolic activity. 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 prenylflavonone 3.3% yield and to modified chalcones in 0.55 and 0.58% yields[14].

Spectroscopic data showing condensation of pyrazole containing substituted hydroxy chalcones.

4-chloro-2-(2-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl) 2,3dihydro 1H-bezodiazepin-4yl-phenol.(R1).

IR (KBr): 1602, 3162 ^{1}H NMR (DMSO-d_6): δ 1.3 (s, 3H, CH_3), δ3.01 (t, 1H, CH), δ 3.5 (d, 2H, CH_2), δ 7.05-8.22 (m, 12H, Ar-H), δ 8.10 (s, 1H, NH), δ 10.8 (s, 1H, OH), ppm; M.S. (m/z): 462.10 (M^+), Anal. Calcd for$C_{25}H_{20}N_4OCl_2$: C, 64.80; H, 4.35; N, 12.09%. Found: C, 64.75; H, 4.33; N, 12.00%.

4-chloro-2-(2-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl) 2,3dihydro 1H-bezodiazepin-4yl-6-iodophenol. (R2).

IR (KBr): 1602, 3162 ^{1}H NMR (DMSO-d_6): δ 0.9 (s, 3H, CH_3), δ 3.1 (t, 1H, CH), δ 3.4(d, 2H, CH_2), δ 7.1-8.0 (m, 11H, Ar-H), δ 8.1 (s, 1H, NH), δ 10.5 (s, 1H, OH), ppm; M.S. (m/z): 588(M^+), Anal. Calcd for$C_{25}H_{19}N_4OCl_2I$: C, 50.96; H, 3.25; N, 9.51%. Found: C, 50.95; H, 3.20; N, 9.41%

2-Bromo-4-chloro-6-(2-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl) 2,3dihydro 1H-bezodiazepin-4yl-phenol. (R3).

IR (KBr): 1602, 3162 ^{1}H NMR (DMSO-d_6): δ 1.2 (s, 3H, CH_3), δ 3.30 (t, 1H, CH), δ 3.5(d, 2H, CH_2), δ 7.0-8.2 (m, 11H, Ar-H), δ 8.0 (s, 1H, NH), δ 11.0 (s, 1H, OH), ppm; M.S. (m/z): 542(M^+), Anal. Calcd for$C_{25}H_{19}N_4OCl_2Br$: C, 55.37; H, 3.53; N, 10.33%. Found: C, 55.30; H, 3.45; N, 10.22%.

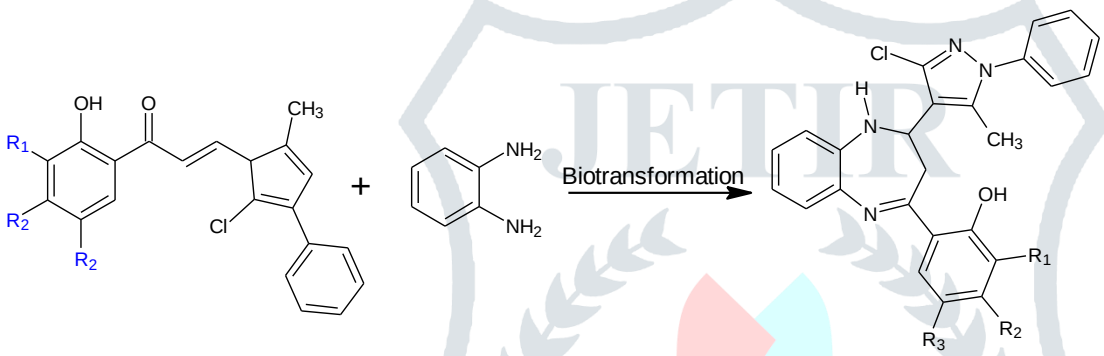

Fig 1: Reaction showing biotransformation of substituted hydroxy chalcones into 1,5 benzodiazepines.

The product which formed in the bio reduction reaction using *Basidiobolus species* was confirmed from spectral analysis is 1,5 benzodiazepines.

Conclusion

Biotransformation is a effective tool for structural modification of biologically active natural and synthetic compounds such as chalcones. In this present study the pyrazole chalcones converted into 1,5 benzodiazepines with better yield. The biotransformation reaction was regioselective, showing condensation reaction which is a part of fungal metabolic activity.

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; 2009 Studies on microbial transformation of albendazole by soil fungi, Indian Journal of Biotechnology, Vol-8: 425-429.

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

[3] Nisar Ahmed, Shikh Abdul Baseer; 2015 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.

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

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

[6] Comasseto, Leandro Helgueira Andrade, Álvaro Takeo Omori; ,2004 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

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

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

[9] Tan Siew Kiat, Richard Pippen, Rohana Yusof, Halijah Ibrahim; 2006 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.

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

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

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

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

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

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

[16] Bhaskar S. Dawane. Shankaraiah G. Konda, Gajanan G. Mandawad, Baseer M. Shaikh; 2010 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 their in vitro antimicrobial evaluation European Journal of Medicinal Chemistry,

Vol 45 387–392,

[17] Sternback, L.H., 1, 4-Benzodiazepines 1971.Angew. Chem., Int. Ed. Engl. 10:34.