ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Microbial transformation of Bioactive Natural Products

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ABSTRACT

In the present study, the fungal biotransformation of 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol (I) is presented. Biocatalysis, isolation and subsequent structure elucidation of the newly transformed metabolite (–)-3-hydroxy -2, 3- dihydro pyrrolo [2, 1-b] quinazoline-9(1H)-one (II) is presented. Microbial transformation of Vasicine (I) by Aspergillus niger, Rhizopus arrhizus, Pencillium notatum and Trematus versicolor afforded (–)-3-hydroxy -2, 3- dihydro pyrrolo [2, 1-b] quinazoline-9(1H)-one (II) (100%) as the sole metabolic product. 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol (I) and (–)-3-hydroxy -2, 3- dihydro pyrrolo [2, 1-b] quinazoline-9(1H)-one (II) screened for antibacterial and antifungal activity.

Keywords: *Aspergillus niger, Rhizopus arrhizus, Pencillium notatum and Trematus versicolor,* microbial transformation.

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ISSN: 0975-8585

INTRODUCTION

Higher plants produce divergent chemicals, such as alkaloids, terpenoids and phenolic compounds, in secondary metabolism. Among these chemicals, alkaloids are very important in medicine because of their high biological activities. Research on microbial and enzymatic biotransformation of commonly available natural compounds into more value added derivatives [1] has always been of interest because of their economical potential to pharmaceutical and food industries [2]. At the molecular level, Nature's catalyst enzymes provide a remarkable enhancement in reaction rates and selectivity over the corresponding unanalyzed reactions [3]. A major selectivity advantage of biocatalysts over traditional systems includes formers ability to exhibit high stereoselectivity [4]. Enzymes provide a means to utilize alternate feed stocks which cannot be selectively activated by conventional catalysts [5].

The medicinal properties of Adathoda *Vasica* Nees (Natural Order: *Acanthaceae*) have been known in India and several other countries for thousands of years. The plant has been recommended by Ayurvedic physicians for the management of various types of respiratory disorders [6]. The leaf extract has been used for the treatment of bronchitis and asthma for many centuries [7]. It relieves cough and breathlessness [8]. The leaves of the plant were found to contain an essential oil and the quinazoline alkaloids vasicine, vasicinone and deoxyvasicine. The roots contain vasicinolone, vasicol and peganine [9]. Research performed over the last three decades revealed that the alkaloids, vasicine and vasicinone present in the leaves, possess respiratory stimulant activity [8].

Vasicine, at low concentrations, induced relaxation of the tracheal muscle. At high concentrations, vasicine offered significant protection against histamine induced bronchospasm in guinea pigs. Vasicinone has been reported to cause bronchodilatory effects both *in vitro* and *in vivo*. Of the two alkaloids, vasicinone was found to be more potent than vasicine, with potential antiasthmatic activity comparable to that of disodium cromoglycate [10].

There have been no reports about the microbial transformation of 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol (I). (–)-3-hydroxy -2, 3- dihydro pyrrolo [2, 1-b] quinazoline-9(1H)-one (II) as a metabolic product has not been obtained by microbial transformation. The present paper is the first report of the production of (–)-3--hydroxy -2, 3- dihydro pyrrolo [2, 1-b] quinazoline-9(1H)-one (II) from microbial transformation of 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol (I) by Aspergillus niger and Rhizopus arrhizus, Pencillium and Trematus versicolor.

For the structure elucidation spectroscopic techniques like UV, GC-MS, , ^{1}H and ^{13}C NMR, and FT/IR were used. Furthermore, antimicrobial activity of substrate (**1**) and the metabolite (2) were evaluated in a micro dilution assay against human pathogenic bacteria and fungi [11,12].

ISSN: 0975-8585

EXPERIMENTAL

Optical rotation was measured on a JASCO DIP-1000 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AM-400 system at 400 and 100 MHz, respectively; δ values were given in Hz. Tetramethylsilane (TMS) at 0.0 ppm was used as internal standard in $CDCl_3$.The Mass spectra were recorded on high resolution mass spectrometer equipped with data system in combination with GC under the following condition, capillary column (30M length 0.25m ID), initial column temperature 80^0C programmed to 250^0 C at the rate of 10^0C/min. The helium as carrier gas with flow rate 1ml/min. IR spectra was recorded on NICOLET-AVATAR 330 FTIR and UV-spectra on UV-spectrometer (Nanodrop- ND1000). The purity of the metabolites were checked by TLC on precoated silica gel GF-254 plates (0.25 mm, Marck)

Isolation of 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol

1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol (I) was isolated from the leaves of Adhathoda vasica. The methanolic extract obtained from the leaves of the plant was partitioned by different pH values. The chloroform extract obtained at pH 10 was loaded on to the silica gel column. The fraction obtained on elution with methanol and chloroform (9:1) further purified crystallization to obtain 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol. Microbial transformation of these compounds were planned in order to obtain more potent derivatives of these compounds. **1**, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol (I) substrate extracted showed characteristic fresh odor, and was brownish yellow amorphous solid with melting point of 205-207^0C.

Thin layer chromatography studies were carried out on pre coated silica gel GF-254 plates. Developing solvents are the mixture of solvents (1, 4 dioxane and dilute ammonia solution 9:1). Chromatogram was observed with ultra violet light at 254 and 366 nm. Also detected using dragendraff's spraying reagent.

Microorganisms

Stock cultures were maintained on agar slants, stored at 4^0C and refreshed every 6 months. Selected fungal organisms Asparagillus niger (16404), Rhizopus arrizus(10260), Pencillium notatum(36740) and Trametus versicolor(20869) known to carry out hydroxylation and oxidation reactions were cultivated at room temperature in 250ml culture flasks containing 100 ml sterile (121^0C, 30 min, autoclaved) medium consisting of $NaNO_3$ (12g) $KHPO_4$ (4.0g) $MgSO_4$ $7H_2O$(2.0g) KCl(2.0g), $FeSO_4.7H_2O$ (0.04g), yeast extract (8.0g) and Glucose (40g) per four litre of media. The medium used for Aspergillus niger was prepared by mixing the following ingredients in distilled water (2.0 ltr), Tartaric acid (7g), Sucrose (10g), KH_2PO_4 (4.0g) $MgSO_4$ H_2O (2.0g) NH_4NO_3 (14.0g) Zn $(OAc)_2$ (0.06g), NaOH per 2 litre of distilled water and pH adjusted at 6.5.

Biotransformation of 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol and isolation:

1, 2, 3, 9-tetra hydropyrrolo [2, 1-b] quinazolin-3-ol (I), a quinazoline type of alkaloid, isolated from Adathoda vasica. It is known to possess many pharmacological activities. Exist

ISSN: 0975-8585

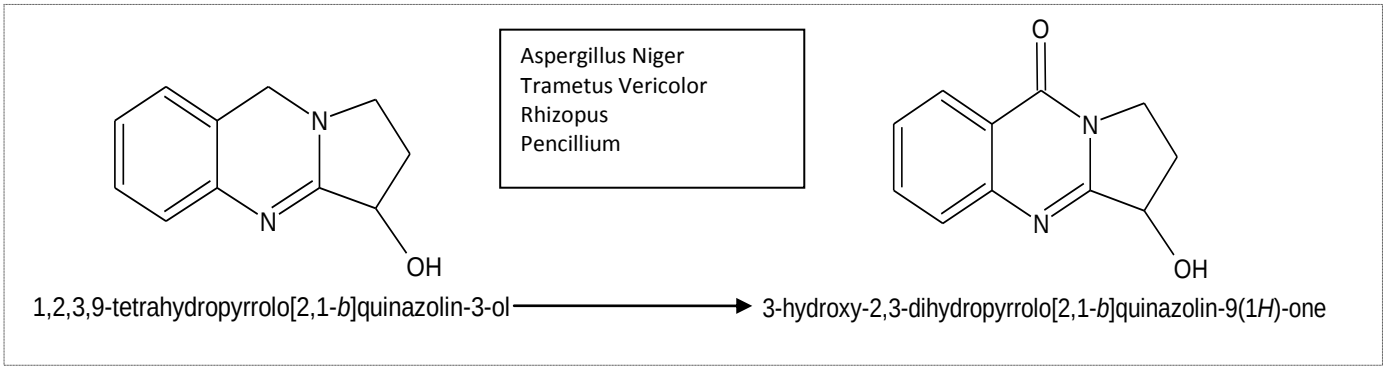
as a raecemic mixture in plant. Only l- form is found to be active. Conversion of this compound into enantiopure derivative and into more potent derivatives may give the better therapeutically active compound in its enantiopure form. This conversion is possible usually by the use of microbial catalyst. Selected compound for microbial conversion is significance in this way. No previous work has been reported on selected substrate. Microbial transformation of the 1, 2, 3, 9-tetra hydropyrrolo [2, 1-b] quinazolin-3-ol (I) were conducted using different fungal species. Expected conversion reactions is to transform the 2, 3, 9-tetra hydropyrrolo [2, 1-b] quinazolin-3-ol (vasicine) to 3-hydroxy -2, 3- dihydro pyrrolo [2, 1-b] quinazoline-9(1H)-one (Vasicinone). (–)-Vasicinone exhibits antitumor, bronchodilating, hypotensive, anthelmintic, and antianaphylactic activities. It is used in The Indian Ayurvedic System of Medicine as a remedy for cold, cough, bronchitis, rheumatism, phthisis and asthma.

Culture flasks were shaken at 150 rpm. After 24–48 h of sufficient growth of the microorganisms, 100 µl of substrate (1, 2, 3, 9-tetra hydropyrrolo [2, 1-b] quinazolin-3-ol) (I) was added to each flask and incubated for 8 days. The biotransformation was stopped by adding EtOAc to the flasks and the broths were combined and extracted with equal amounts of EtOAc successively. After filtration of the mycelia, the extracts were dried over anhydrous sodium sulfate concentrated and dried over anhydrous sodium sulphate and concentrated the crude product which was purified by column chromatography with petroleum ether: ethyl acetate (2:8). Preparative TLC was carried out using 1, 4 dioxane: dilute ammonia as developing solvents to initial identification of compound II. Culture controls consisted of fermentation blanks in which the microorganism was grown under identical conditions but without the addition of substrate. After 8 days of incubation, controls were also harvested and analysed by TLC.

Gas chromatography mass spectrometry (GC-MS) conditions

The samples were analyzed and screened by GC-MS using a Thermo system column (30 m x 0.25 mm i.d., 0.25 µm film thickness) was used with helium as a carrier gas (1.5 ml/min). GC initial column oven temperature was kept at 80^0C and programmed to 250 ^{0}C at a rate of 10^0 C/min, and then kept constant at 230^0C for 11 min. Injection was carried out in split less mode. The injector temperature was at 250^0C. Mass spectra were recorded at 70 eV and mass range was from m/z 35 to 425. Library search was carried out using the in-house library “nest Library of organic compounds” and in comparison with authentic samples.

Scheme



ISSN: 0975-8585

Figure1: The biotransformation of 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol Compound I) and (–)-3-hydroxy -2, 3- dihydro pyrrolo [2, 1-b] quinazoline-9(1H)-one (Compound II) in 4days

Spectral Data

3-hydroxy-2,3-hydropyrole[2,1-b]quinozolone-9(1H)-one Yield 5.9% ([α] 23D 97=0.9) UV (MeOH) max log(ε) 273nm. IR (nujol) vmax 3169, 1683, 1463cm-1. H1NMR (CDCl3 200MHz) δ 2.20-2.45(m.1H), 2.60-2.80 (m, 1H) .3.90-4.15 (m, 1H) 4.30-4.15 (m, 1H), 5.27 (H), 7.40-7.60 (m, 1H), 7.65- 7.85 (m,2H) 8.31 (d,J=6H=1H) ^{13}CNMR (CDCl3, 50 MHz) δ 29.4, 43.5, 72.0, 121.1, 126.7, 126.8, 134.4, 148.6, 160.1 , 160.6, MS (m/e) 202, 185, 174, 146, 130, 119, 102, 90, 76, 63, 55. Anal: C65.34: H 4.99: N13.85 calculated for $C_{11}H_{10}N_2O_2$, Found C. 65.18, H 5.06; N 13.77.

Antimicrobial assay

Microdilution broth susceptibility assay (Iscan *et al.*, 2002) was used for the antimicrobial evaluation of the substrate and metabolite. Stock solution was prepared in dimethylsulfoxide (DMSO) and dilution series were prepared up to 0.9μg/ml using sterile distilled water in 96 well microtiter plates. Overnight grown microbial suspensions in double strength Mueller-Hinton broth and suspension of *Candida albicans* in yeast medium were standardized to approximately 108 CFU/ml. 100 μl of each microbial suspension was then added to each well. The last row containing only the serial dilutions of antimicrobial agent without microorganism was used as negative control. Sterile distilled water and medium served as a positive growth control. After incubation at 37^{0}C for 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC) expressed both in μg/ml and mm, as seen in Table I. Chloramphenicol was used as standard antibacterial agent for the bacteria, whereas ketoconazole was used for *C. albicans* [13].

RESULTS AND DISCUSSION

1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol (**1**) was incubated with the selected fungal species for 8 days. Preliminary TLC screening showed a new metabolite at the fourth day. It was confirmed by GC-MS analysis. This biotransformation reaction has depicted in figure. 1. This compound was purified from the microbial transformation broth using the EtOAc extract *via* column chromatography. Apolar to polar gradient elution starting from *n*-hexane allowed the isolation of pure crystals which were subjected to further spectroscopic analyses. The ^{1}H NMR spectrum showed at *δ 2.*3 a proton demonstrating that there was no change at position 1, but at position 9, where the proton was oxidized to a keto group. In conclusion, we have achieved the biotransformation of 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol in only one step in overall good yield. The key-step for the reaction is the regioselective biotransformation. The advantage of this method is the access to both bioactive molecule, (–)-3-hydroxy -2, 3- dihydro pyrrolo [2, 1-b] quinazoline-9(1H)-one from readily available starting materials in only one-steps using a common protocol. The methodology can be extended to the synthesis of other natural products and their mimics to prove its generality. To the best of our knowledge, the

isolation and absolute configuration of 9-keto-(-)-vasicine(II) *via* biotransformation is reported with this work for the first time.

The substrate (–)-vasicine (I) as well as the metabolite 2 were subjected to a microdilution broth assay (Iscan *et al.*, 2002) against various gram positive and negative human pathogenic bacteria and the yeast *Candida albicans.* The metabolite showed good inhibitory activity compared to vasicine and results were comparable with the standard antimicrobial agents.

Table I. Antimicrobial activity of Biotransformation of 1, 2, 3, 9-tetra hydrpyrrolo [2, 1-b] quinazolin-3-ol (Compound I) and its metabolite (Compound II)

Microorganism	Source	Compound (1)	Compound II	Std
Escherichia coli	ATCC 25922	125	125	62.5
Staphylococcus aureus	ATCC 6538	250	62.5	125
Pseudomonas aeruginosa	ATCC 27853	250	250	250
Candida albicans	ATCC 10231	250	125	125

MIC values are given in both μg/ml. Std.: Chloramphenicol-antibacterial. * Ketoconazole- antifungal.

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