Asian Journal of Chemistry Vol. 19, No. 1, (2007), 539–542

10*H*-Indolo[3,2-b]quinoline, a Potent Brine Shrimp Toxin from *Justicia betonica*

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10*H*-Indolo[3,2-b]quinoline, an alkaloid from *Justicia betonica,* exhibited a very potent brine shrimp lethality, but no significant anti-oxidative or 5-lipoxygenase inhibitory activities.

Key Words: 10*H*-Indolo[3,2-b]quinoline; *Justicia betonica;* Brine shrimp lethality.

INTRODUCTION

About fifty *Justicia* (Family Acanthaceae) species have been recorded in India and ten species are used in the indigenous systems of medicine, the Ayurveda and the Unani. *Justicia betonica* is an ornamental plant commonly grown in Northeast of Thailand, Sri Lanka and in India[1, 2]. Its flowers are used in swelling, as antidiarrhoea and an antiinflammatory agent in India and its leaves are used as poultice for boils in Sri Lanka[1]. During the course of our studies on *Justicia betonica,* we have isolated 10H-quindoline (**1**) as a major alkaloid and 6H-quinindoline, 5H,6H-quinindolin-11-one and a new alkaloid, jusbetonin, as minor components from the leaves[3]. We have screened compound **1** for various biological activities and found that it is a potent brine shrimp toxin and the details are presented in this note.

EXPERIMENTAL

Isolation and characterization of compound **1** was reported earlier[3]. Artemia saline cysts and sea salt were obtained from the aquarium stores. Nitroblue tetrazolium (NBT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), xylenol orange (3,3′-bis[N,N-bis(carboxymethyl)aminomethyl]-*o*-cresolsulfonephthalein tetrasodium salt), linoleic acid and gallic acid were obtained from Sigma Chemical Company, U.S.A. Other Chemicals and reagents used were AR grade and were obtained from Qualigens Fine Chemicals, India. Podophyllotoxin and 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA) were kindly provided by M/s Laila Impex, Vijayawada. Griseofulvin and ciprofloxacin were obtained from M/s Venkat Pharma, Vijayawada. *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epider-*

mis, Staphylococcus aureus and *Aspergillus niger* were obtained from National Chemical Laboratory, Pune, India.

Determination of brine shrimp lethality: Brine shrimp (*Artemia salina*) napulii were hatched using brine shrimp eggs in a conical shaped vessel of 1 L volume filled with sterile artificial sea water (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1 N NaOH) under constant aeration for 48 h. After hatching, 10 nauplii were drawn through a pipette and placed in each vial containing 4.5 mL sea water and added various concentrations of drug solutions and volume was made up to 5 mL using brine solution and maintained at 37°C for 24 h under the light of incandescent lamps and surviving larvae were counted. Each experiment was conducted along with control (vehicle treated) at various concentrations of the test substance in each set that contained 6 tubes and the average results are reported. The percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. LC_{50} values were obtained by probit analysis using Finney software[4].

Determination of superoxide radical scavenging activity: Superoxide radical scavenging activity was determined by the method of McCord and Fridovich[5]. The assay mixture contained aqueous solutions of EDTA (6.0 mM), NaCN (3 μg), riboflavin (2 μM), and NBT (50 μM), various concentrations of the test substances in methanol and a phosphate buffer (58 mM, pH 7.8) in a final volume of 3 mL. The tubes were shaken well and the absorbance was measured at 560 nm. The test tubes were uniformly illuminated with an incandescent lamp for 15 min, after which the absorbance was measured again at 560 nm. The per cent inhibition of superoxide radical generation was measured by comparing mean absorbance values of the control and those of the test substance. IC_{50} values were obtained from the plot drawn of concentration in μg *vs.* percentage inhibition and were converted into μM. Gallic acid was used as a standard.

Determination of DPPH free radical scavenging activity: DPPH radical scavenging activity was measured based on the reduction of methanolic solution of the coloured DPPH[6]. The reaction mixture contained 1×10^{-4} M methanolic solution of DPPH and various concentrations of test substance in methanol and kept in a dark area for 50 min. The absorbance of the samples was measured on a spectrophotometer at 517 nm against a blank. The difference in the absorption of DPPH solution and those of the samples is inversely proportional to the free radical scavenging ability of the test substance. The per cent inhibition was determined by comparing the mean absorbance values of control tubes (mean control OD is 0.8998) and those of the test substance. IC_{50} (50% inhibitory concentration) values were obtained from the plot drawn of concentration in μM *vs.* percentage inhibition. Ascorbic acid was used as a standard.

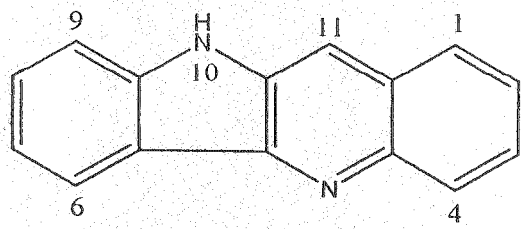
Determination of antibacterial and antifungal activities: The antibacterial activity was determined by agar cup-plate (cup dia.: 8 mm) diffusion method[7] against the organisms *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermis* and *Staphylococcus aureus* at different concentrations of 500, 200, 100 and 50 μg/0.05 mL. Ciprofloxacin was used as standard. The antifungal activity was also determined by a similar procedure[7] against *Aspergillus niger* using griseofulvin as standard.

Determination of 5-lipoxygenase enzyme inhibitory activity: The compound was screened for 5-lipoxygenase enzyme inhibitory potential using colourimetric method, at different concentrations. The assay mixture contained 50 mM phosphate buffer pH 6.3, 5-lipoxygenase, various concentrations of test substances in dimethyl sulphoxide and linoleic acid (80 mM) in a total volume of 0.5 mL, after 5 min incubation of above reaction mixture, 0.5 mL ferric xylenol orange reagent (in perchloric acid) was added and absorbance was measured after two min at 585 nm on a spectrophotometer. Controls were run along with test in a similar manner except using vehicle instead of test substance solution. Percent inhibition was calculated by comparing absorbance values of test solution with that of control. All the tests were run in triplicate and averaged. 3-*O*-Acetyl-11-ketoboswellic acid (AKBA) was used as a standard.

RESULTS AND DISCUSSION

Brine shrimp lethality has been used as a "bench top bioassay" for the discovery and purification of bioactive natural products[8, 9]. Freeze-dried cysts of brine shrimp (*Artemia* species) are readily available at aquarium stores. The cysts last for several years and can be hatched without special equipment[10]. The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity and the results obtained by this assay have been reported to be corroborative with cytotoxicities determined by 9 KB and 9 PS cell lines[4, 11]. Based on the results of this preliminary assay, detailed cytotoxicity and anti-tumour activities of the extracts and the pure compounds could be undertaken.

10*H*-Indolo[3,2-b]quinoline (**1**) showed potent brine shrimp lethality (LC_{50}: 1.50 μg/mL) compared to standard podophyllotoxin (LC_{50}: 3.15 μg/mL)



10*H*-Indolo[3,2-b]quinoline (**1**)

Antioxidant activity of compound **1** was evaluated by superoxide radical scavenging (NBT method)[5] and the DPPH[6] free radical scavenging methods. Compound **1** did not exhibit significant superoxide free radical (IC_{50}: > 50 μM/mL) or DPPH free radical (IC_{50}: > 50 μM/mL) scavenging activities.

The antibacterial activity of **1** was determined against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus epidermis* and *Staphylococcus aureus* and antifungal activity against *Aspergillus niger*. No significant antibacterial or antifungal activity was observed for **1** even at high doses of 500 μg.

5-Lipoxygenase enzyme inhibitory activity is an important method for screening anti-inflammatory activity. 5-Lipoxygenase enzyme inhibitory activity of **1** was determined using a colorimetric method[12] and at different concentrations. Percentage inhibition was determined by comparing the absorbance values of test

and control tubes. IC_{50} values were obtained from the plot drawn for concentration *vs.* percentage inhibition. Compound **1** did not exhibit significant 5-lipoxygnase inhibitory activity (IC_{50}: > 250 μg/mL).

ACKNOWLEDGEMENTS

The authors thank Sri G. Gangaraju, the Chairman, Laila Group, Vijayawada for encouragement, Mr. G. Rama Raju, Director, Laila Impex for providing podophyllotoxin and AKBA standards and Mr. A.V. Krishnaraju for helpful discussions.

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(*Received*: 10 December 2005; *Accepted*: 24 June 2006) AJC-4974