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ORIGINAL RESEARCH ARTICLE

Synthesis of novel anticancer derivatives of a rare phytocompound scillascillin from a new species *Ledebouria hyderabadensis*

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ABSTRACT

*Ledebouria is a genus of deciduous or weakly evergreen bulbs in the Hyacinthaceae family. This is recognized as the first collection made of the new taxon Ledebouria hyderabadensis. The compound Scillascillin **1** is a rare and novel benzocyclobutene ring containing homoisoflavanone, isolated for the first time from L. hyderabadensis. In vitro anticancer activity performed using MTT assay, on compound **1** and its semi synthetic derivatives showed significant inhibitory activity against human cancer cell lines A549 (Adenocarcinoma), HepG2 (Hepatocellular carcinoma), MCF-7 (breast cancer),SKOV3 (Ovarian Adenocarcinoma)and HeLa(Immortalised). Amongst the derivatives, compound **4** showed better anti-cancer activity against HepG2 (IC_{50}: 61.34 ± 0.31 µM) when compare with parent Scillascillin **1**(IC_{50}: 244.69 ± 0.01 µM).*

INTRODUCTION

Natural products have played a key role in human medicine as many of them are either used as direct or derivatives thereof. Indeed, it is estimated that about 40% of all medicines is either natural products or their semi-synthetic derivatives (Jacob, 2009). Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines such as aspirin, digitoxin, morphine, quinine, and pilocarpine (Butler, 2004). Natural products research continues to explore a variety of lead structures, which may be used as templates for the development of new drugs by the pharmaceutical industry (Patwardhan et al., 2004).These approved substances, representative of very wide chemical diversity, continue to demonstrate the importance of compounds from natural sources in modern drug discovery efforts (Chin et al., 2006).

Ledebouria is a genus of deciduous or weakly evergreen bulbs in the Hyacinthaceae family. Our earlier phytochemical investigation on the underground bulbs of the newly discovered species *L. hyderbadensis* resulted in the isolation of a rare homoisoflavone-Scillascillin which exhibited significant cytotoxic activity against selected human cancer cell lines in *in vitro* mode (Yakaiah et al., 2014). Different types of homoisoflavones reported from *L.graminifolia* also showed potential cytotoxic activity (Mutanyatta et al., 2003). Homoisoflavonoids are a class of naturally occurring oxygen containing heterocyclic compounds. Both natural and synthetic homoisoflavonoids exhibit numerous biological

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activities (Qualig et al., 1999; Desideri et al., 1997; Siddaiah et al., 2006; Yakaiah et al., 2014; Dai et al., 2013; Das et al., 2009; Guo et al., 2013; Gupta et al., 2009) like antifungal, hypocholesterolemic, antimutagenic, antirhinovirus, antiallergic, antihistaminic, anti-inflammatory, antioxidant, antiviral, inhibition of platelet aggregation etc. The present paper reports the semi synthesis some novel derivatives of Scillascillin and checking their anticancer potentials against different human cancer cell lines.

EXPERIMENTAL SECTION

General

Melting points of all the compounds were recorded on KRUSS (Made in Germany) melting point apparatus and wereuncorrected. IR spectra were recorded on a Perkin–Elmer FT-IR 240-C spectrometer using KBr pellets. NMR spectra were recorded on Bruker Avance 300 MHz in $CDCl_3$ using TMS as internal standard. Electron impact (EI) and chemical ionization mass spectra were recorded on a VG Micro mass model 7070H instrument. All the reactions were monitored on silica gel precoated TLC plates of Merck and spots were visualized with UV light. Silica gel (100-200 mesh) used for column chromatography was procured from Merck.

Plant material collection

The underground bulb part of *L. hyderabadensis* was collected at Osmania University Campus, Hyderabad, India during mid-rainy season, and was identified by taxonomist, Dr. Venkat Ramana, Assistant Professor, Department of Botany, Nizam College, Osmania University, Hyderabad, India. A voucher specimen (CIMAP-SH/11) was deposited at CSIR-Central Institute of Medicinal and Aromatic Plants, Research Center, Hyderabad.

Extraction and isolation

Fresh underground bulbs were collected, shade dried andpowdered (1.5 kg). The powdered material was extracted with methanol at reflux temperatures using Soxhlet apparatus (3 L, 24 siphon cycles). The extract was evaporated at reduced pressures (at 40°C) and then freeze-dried. The viscous gummy methanol extract was washed several times (at room temperature) with *n*-hexane to remove fats and other coloring impurities. The resulting semi-solid gummy residue was then subjected to column chromatography over silica gel (100-200 mesh) and elution of the column with 10% ethylacetate in hexane solvent mixture yielded a pure pale yellow colored compound **1** (Yield: 0.01%, Wt: 150 mg, Thin-Layer Chromatography 30:70 ethyl acetate: hexane, Rf 0.52, Melting point: 194°C)

Synthesis of compound (2) general procedure

Scillascillin (**1**, 30 mg) along with potassium carbonate was taken in 10 mL of dry DMF and later Propargyl bromide was added slowly while stirring. The reaction mixture was stirred at room temperature for 4 h. To the reaction mixture, added excess of ethyl acetate and washed with water (70 mL x 3). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure. This crude residue was purified by column chromatography over silica gel (100-200 mesh) with 25% ethyl acetate in hexane to get compound **2** in pure state.Light brown solid, yield: 93 %, mp: 205–206 °C, IR (KBr, cm^{-1}):3279 (C–H), 3058 (Ar–H), 2922 (C–H), 2112 (Ca"C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.55-2.56 (1H, t, H-5^{113}), 2.63-2.64 (1H, t, H-7^3), 2.85-2.85 (1H, d, *J* = 13.8 Hz, H-9), 3.57-3.60 (1H, d, *J* = 13.8 Hz, H-9), 4.55(2H, s, H-5^{111}), 4.74 (2H, s, H-7^1), 5.84-5.85 (2H, d, *J* = 1.25 Hz, H-2), 5.87-5.88 (2H, d, *J* =1.0 Hz, O-CH_2-O),6.26-6.27 (1H, d, *J* = 2.5 Hz, H-6), 6.32-6.33 (1H, d, *J* = 2.5 Hz, H-8),6.55 (1H, s, H-2^1),6.69 (1H, s, H-5^1).^{13}C NMR ($CDCl_3$, 75 MHz) δ: 34.2 (C-9), 54.7 (C-3), 56.1 (C-5^{111}), 56.7 (C-7^1), 73.6 (C-2), 76.5 (C-5^{113}), 76.6 (C-7^3), 77.5 (C-5^{112}), 77.7 (C-7^2), 95.2 (C-8), 95.5 (C-6), 100.1 (O-CH_2-O), 104.2 (C-4a), 105.8 (C-5^1), 105.9 (C-2^1), 134.8 (C-1^1), 136.4 (C-6^1), 146.8 (C-3^1), 148.1 (C-4^1), 160.4 (C-8a), 163. 4 (C-5), 164.8 (C-7), 189.0 (C-4).

Synthesis of compound (3) general procedure

Scillascillin (**1**, 25 mg) along with potassium carbonate was taken in 15 ml of dry DMF and later 4-Nitrobenzylbromide was added slowly while stirring. To the reaction mixture, added excess of

ethyl acetate and washed with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure. This crude residue was purified by column chromatography over silica gel (100-200 mesh) in 30% ethyl acetate in hexane to get compound **3** in pure state.yield: 83 %, ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 3.00-3.04 (1H, d, *J* = 13.8 Hz, H-9), 3.54-3.58 (1H, d, *J* = 13.8 Hz, H-9), 4.53-4.56 (1H, d, *J* = 11.54 Hz, H-2), 4.58-4.60 (1H, d, *J* =11.54 Hz, H-2),5.23 (2H, s, 7^1), 5.9-5.9 (1H, d, *J* =1.50 Hz, O-CH_2-O), 5.92-5.93 (1H, d, *J* =1.0 Hz, O-CH_2-O),6.12-6.13 (1H, d, *J* = 2.5 Hz, H-6), 6.15-6.16 (1H, d, *J* = 2.5 Hz, H-8),6.61 (1H, s, H-2^1),6.74 (1H, s, H-5^1), 7.6-7.62 (1H, d, *J* = 9.0 Hz, H-7^2), 8.30-8.31 (2H, d, *J* = 9.0 Hz, H-7^3), 12.1(1H, s, 5-OH).

Synthesis of compound (4) general procedure

Scillascillin (**1**, 20 mg) was dissolved in pyridine and to this mixture, acetic anhydride was added slowly while stirring at room temperature under nitrogen atmosphere for 12 h. Later, the solvent was removed under reduced pressure and the residue was diluted with cold distilled water and extracted thrice with ethyl acetate. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to get the crude product. The crude was purified by column chromatography with ethyl acetate in hexane to give a pure compound **4** (5 mg) as a colourless amorphous powder, yield: 74 %, ^{1}H NMR ($CDCl_3$, 300 MHz) δ : 2.33 (3H, s, AcO), 3.04-3.07 (1H, d, *J* = 13.5 Hz, H-9), 3.54-3.57 (1H, d, *J* = 14.0 Hz, H-9), 4.55-4. 58 (1H, d, *J* = 11.54 Hz, H-2), 4.61-4.64 (1H, d, *J* =11.8 Hz, H-2), 5.90-5.91 (1H, d, *J* =1. 50 Hz, O-CH_2-O),5.93-5.94 (1H, d, *J* =1. 5 Hz, O-CH_2-O),6.31-6.32 (1H, d, *J* = 2.26 Hz, H-6), 6.33-6.34 (1H, d, *J* = 2.26 Hz, H-8),6.63 (1H, s, H-2^1),6.75 (1H, s, H-5^1), 11.9 (1H, s, 5-OH).

Anticancer activity

Cell culture

Human cancer cell lines A549 (Adenocarcinoma), HepG2 (Hepatocellular carcinoma), MCF-7 (breast cancer),SKOV3 (Ovarian Adenocarcinoma) and HeLa (Immortalised) were obtained from American Type Culture Collection (Manassas, VA, USA) the cell lines were grown in Dulbecco's modification of Eagle's medium medium supplemented with 10% fetal bovine serum, 0.3% sodium bicarbonate, 10 mL/L antibiotic anti-mycotic solution (10,000 U/ml penicillin, 10 mg/L streptomycin, and 25 µg/mL amphotericin B), 1 mL/L of 4 mM L-glutamine and 1 mL/L of 100 mM sodium pyruvate culture was maintained in CO_2 incubator at 37°C with a 90% humidified atmosphere and 5% CO_2.

Preparation of samples for 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide assay

Test compounds were taken in 10 mg/ml of DMSO and various dilutions were made with sterile phosphate buffered saline (PBS) (×1) to get desired concentrations. All formulations were filtered with 0.22 µm sterile filter and 20 min of ultraviolet (UV) eradication before adding to the 96 well plates containing cells.

Cytotoxicity evaluation (3- (4,5- dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide assay)

Cytotoxicity of formulations was assessed using MTT assay to determine the cell viability according to a reported method (Hansen et al., 1989).The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells into purple formazan crystals, which gets dissolved in dimethyl sulfoxide (DMSO) and read at 570 nm. Briefly, 1 X 10^4 exponentially growing cells were seeded into each 96 well plate (counted by Trypan blue exclusion dye method) allowed to grow until 60-70% confluence then compounds (name of the compounds if applicable) were added to the culture medium with the final concentrations ranging from of 10, 25, 50, and 100 µg/mL and along with controls [negative (without compound) and positive (doxorubicin)] incubated for 24 h CO_2 incubator at 370°C with a 90% humidified atmosphere and 5% CO_2. Later, the media of the wells were replaced with 90 µL of fresh serum free media and 10 µL of MTT (5 mg/mL of PBS), plates were incubated at 37°C for 2 h, there after the above media was discarded and allow to dry for 30 min. Add 100 µL of DMSO in each well at 37°C for 5 min. The purple formazan crystals were dissolved and immediately

read absorbance at 570 nm was measured using Spectra Max plus 384 UV-Visible plate reader (Molecular Devices, Sunnyvale, CA, USA). Inhibitory concentration IC_{50} values were determined by probit analysis software package of MS-excel, % cell viability (from control) versus concentration.

RESULTS AND DISCUSSION

Chemical modification of Scillascillin **1** resulted in the generation three novel derivatives (**Scheme I**). Compound **1** treated with propargyl bromide in presence of potassium carbonate at room temperature to obtain 5,7-dipropynoxy derivative **2**. Compound **3** was obtained by an interaction of 4-nitro-benzyl bromide with **1** in presence of potassium carbonate at room temperature. Compound **4** (7-O-acetyl-scillaschillin) was prepared initially by dissolving compound **1** in pyridine and to this, acetic anhydride was added slowly while stirring at room temperature under nitrogen atmosphere for 12 h. All the semi-synthesized compounds were characterized by spectroscopy such as IR, ^{1}H &^{13}C NMR.

In the ^{1}H NMR, compound **2** showed two singlet resonances at δ 4.55(2H, s, H-5^{111}) and 4.74 (2H, s, H-7^{1}) ppm due to two methylene bridge protons and the corresponding carbon resonances, in ^{13}C NMR spectra, were appeared at δ 56.1 (C-

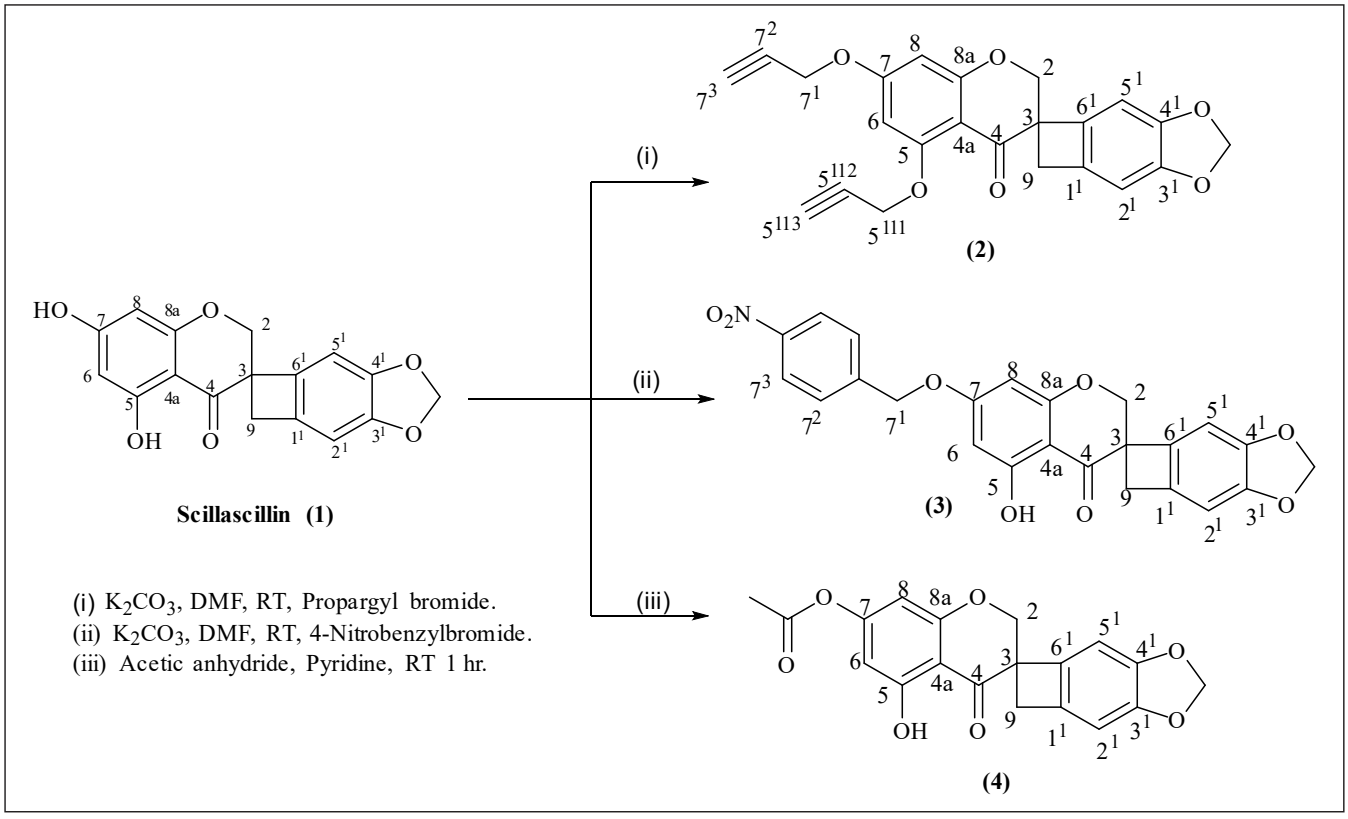

Figure 1. Semi-synthetic derivatives of Scillascillin

Table 1: *In vitro* anticancer activity of compound 1 and semi-synthetic derivatives

Sample Code	IC_{50} in µM				
	A549	HepG2	MCF-7	HeLa	SKOV3
1	64.11±0.033	244.69±001	1667.62±0.012	487.35±0.21	781.32±0.29
2	26.82±0.11	32.31±004	933.10±0.13	410.15±0.10	210.27±0.01
3	2931.70±0.2	774.81±0.12	1235.48±0.41	1247.89±0.23	3214.24±0.10
4	426.14±0.13	61.34±0.31	579.46±0.25	870.29±0.41	641.19±0.04
Doxorubicin	1.84±0.120	4.21±0.14	17.99±0.11	29.21±0.10	36.84±0.24

5^{111}), 56.7 (C-7^{1}). Compound **3** showed a singlet proton resonance at δ 5.23 (2H, s, 7^{1}) ppm due to methylene protons and compound **4** showed a singlet resonance at δ 2.33 (3H, s, Acetate) ppm was due to methyl protons. In the IR spectra, the aliphatic C–H stretching of methylene group in compounds **2**, **3** and **4** appeared at 2,900–2,960 cm^{-1}, while the aromatic C–H stretching frequencies were observed between 3,000 and 3,130 cm^{-1}. Spectral data for all the new compounds were presented in the experimental section. *In vitro* anticancer activity performed using MTT assay showed compound **1** and its semi synthetic derivatives as significantly active (**Table I**) against human cancer cell lines A549 (Adenocarcinoma), HepG2 (Hepatocellular carcinoma), MCF-7 (breast cancer),SKOV3 (Ovarian Adenocarcinoma) andHeLa (Immortalised). Compound **2** showed moderate anticancer activity against A549 (IC_{50}: 26.82 ± 0.11 ìM), HepG2 (IC_{50}: 32.31±0.04 ìM), MCF-7 (IC_{50}: 933.10±0.13 ìM), HeLa(IC_{50}: 410.15±0.10 µM), SKOV3(IC_{50}: 210.27±0.01 µM) whereas, compound **4** showed better anti-cancer activity against HepG2 (IC_{50}: 61.34±0.31 µM) when compared to the parent Scillascillin **1**(IC_{50}: 244.69 ± 0.01 µM).

CONCLUSIONS

Compound Scillascillin was isolated for the first time from the newly discovered underground bulbs of *Ledebouria hyderabadensis* in reasonably good yield and three novel derivatives of Scillascillin were prepared and screened for their anticancer activity. The natural product Scillascillin showed comparable *in-vitro* anticancer activity with standard drug doxorubicin. Induction of propargyl moiety on 7, 5 hydroxyl position in 2 enhanced the anticancer activity in few cell lines whereas induction of other aromatic and aliphatic groups at 7-hydroxyl position (3 and 4) did not improve the activity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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