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Synthesis of novel anticancer iridoid derivatives and their cell cycle arrest and caspase dependent apoptosis

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ABSTRACT

Nyctanthes arbortristis Linn (Oleaceae) is widely distributed in sub-Himalayan regions and southwards to Godavari, India commonly known as Harsingar and Night Jasmine. In continuation of our drug discovery programme on Indian medicinal plants, we isolated arbortristoside-A (**1**) and 7-O-trans-cinnamoyl 6β-hydroxyloganin (**2**) from the seeds of *N. Arbortristis*, which exhibited moderate in vitro anticancer activity. Chemical transformation of **2** led to significant improvement in the activity in derivative **8** and **15** against HepG2 (human hepatocellular carcinoma), MCF-7 (breast adenocarcinoma) cell lines. The compounds **8** and **15** were also capable of cell cycle arrest and caspase dependent apoptosis in HepG2 cell lines. These iridoid derivatives hold promise for developing safer alternatives to the marketed drugs.

Introduction

Iridoids represent the group of cyclopentan – (c) – pyran monoterpenoids. They are found as natural constituents in a large number of plant families. Iridoids were first isolated in the latter part of the nineteenth century, but it was not until 1958 that Halpern and Schmid (Halpern et al. 1958) proposed the basic skeleton of the iridoids in their investigation of the structure of plumieride, which possess various biological activities (Chopra et al. 1956). The name iridoid is a generic term derived from the names iridomyrmecin, iridolactone and iridodial compounds isolated from some species of Iridomyrmex, a genus of ants, in which they occur as defensive secretions (Roth and Eisner 1962). These compounds have been referred to as pseudoindicans. They have also been referred to as aucubin glucosides.

Arbortristoside-A (**1**) and 7-O-trans-cinnamoyl-6β-hydroxyloganin (**2**) belongs to iridoid class of compounds are the major bioactive compounds of numerous herb species such as *Nyctanthes arbortristis* Linn (Division: Magnoliophyta; Class: Magnoliopsida; Order: Lamiales; Family: Oleaceae), commonly known as Harsingar and Night Jasmine and possesses leishmanicidal (Tandon et al. 1991), antiplasmodial (Tuntiwahwuttikul et al. 2003), antispermatogenic (Gupta et al. 2006), antiallergic (Gupta et al. 1995), anti-inflammatory (Amrite et al. 2006; Patel et al. 1998; Saxena et al. 1984; Sanjita Das et al. 2008), antinociceptive (Sanjita Das et al. 2008) and analgesic activity (Saxena et al. 1987).

In continuation of our drug discovery programme on anticancer agents from Indian medicinal plants, we isolated large quantities of 7-O-trans-cinnamoyl-6β-hydroxyloganin (**2**) from the seeds of *Nyctanthes arbortristis* Linn and planned to carry out chemical transformation to improve its therapeutic application. Chemical transformation of bioactive compounds of medicinal herbs is one of the most common approaches in drug discovery to improve the therapeutic properties. For example the anticancer drugs teniposide and etoposide are derivatives of podophyllotoxin and topotecan and irinotecan are analogues of camptothecin, which have better therapeutic benefits than the parent natural products. Towards this goal, we have synthesized novel derivatives of compound **2** and evaluated their anticancer activity against HepG2 (human hepatocellular carcinoma), MCF-7 (breast adenocarcinoma), MDAMB-231 and NIH/3T3 cell lines (Table 1). Further we have studied the apoptosis inducing ability, effect on cell cycle and caspase-3 activation studies of most active compounds **8** and **15**.

Materials and methods

General chemistry

IR spectra were recorded on Perkin-Elmer RX-1 spectrometer. Using either KBr pellets (or) in neat. ^{1}H NMR, ^{13}C NMR, DEPT-90 and DEPT-135 spectra were run on Bruker Advance DPX 300 MHz and 200 MHz in $CDCl_3$. Chemical shifts are reported as values in

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Table 1
Chemical structure and in vitro anticancer activity (IC_{50} in μM) of compounds **1**, **2** and its derivatives (**3**–**15**).

Comp. No.	HepG2	MDAMB-231	MCF-7	NIH/3T3
1	89	80	87	78
2	78	76	79	84
3	469	270	310	280
4	134	125	147	140
5	113	97	98	120
6	42	49	45	55
7	56	45	44	59
8	12	10	14	19
9	145	156	151	167
10	79	83	87	92
11	74	82	76	89
12	23	16.8	32	34
13	78	82.6	76	93
14	29	32	37	45
15	14	18	16	31
Epirubicin	4.6	7.7	3.7	3.2
Paclitaxel (PTX)	2.1	1.24	4.6	3.6
Doxorubicin	1.24	3.26	3.84	4.57

ppm relative to $CHCl_3$/DMSO with TMS as internal standard. ESI mass spectra were recorded on JEOL SX 102/DA-6000. Plates for thin layer chromatography (TLC) were prepared from silica gel 60 GF254 (Merck) and activated by drying at 100 °C for 2 h. Chromatography was executed with silica gel (60–120 mesh) using mixtures of chloroform, methanol and hexane as eluants. Visualization was obtained under UV light and spraying with 10% sulphuric acid in methanol.

Background of plant

Nyctanthes arbortristis Linn (Oleaceae), commonly known as Harsingar and Night Jasmine. It is a shrub growing to 10 m tall, with flaky grey bark. The fruit is a flat brown heart-shaped to round capsule 2 cm diameter, with two sections each containing a single seed. Which have been claimed to possess multiple pharmacological activities like antibacterial, antifungal, anti-influenza, anti-inflammatory, analgesic, antipyretic, antihistaminic, antiulcer, hypnotic, tranquilizing, hepatoprotective, antidiabetic, antianemic, immunobioactivities, antioxidant, antispermatogenic, etc. (Rachna and Mridula 2011).

Collection of medicinal plant

N. arbortristis Linn seeds were purchased from the local market of Lucknow, India and the authentification was done by Botany Division of Central Drug Research Institute, Lucknow and is kept in the herbarium for future reference.

Extraction

Powdered *N. arbortristis* Linn seeds (4 kg) were placed in glass percolator with 95% ethanol (10 l) and allowed to stand for 24 h at room temperature. The percolate was collected and these processes were repeated for four times. The combined percolate was evaporated under reduced pressure at 50 °C to afford ethanol extract. The weight of extract was found to be 300 g.

Fractionation

The ethanol extract was macerated with hexane. The hexane soluble fraction was separated and evaporated under reduced pressure to afford hexane fraction (F001, 80 g). Chloroform was added to hexane insoluble portion, and the resultant solution was evaporated under reduced pressure to afford chloroform fraction (F002, 120 g). *n*-Butanol was added to chloroform insoluble portion, the *n*-butanol soluble fraction was evaporated under reduced pressure at 60 °C afford *n*-butanol fraction (F003, 80 g).

Isolation and purification of Iridoids

n-Butanol fraction (80 g) was chromatographed on a column of silica gel (60–120 mesh) and eluted with chloroform and methanol in increasing polarity. Fractions were collected and then combined on the basis of TLC pattern to get two subfractions (A and B). Fraction A was rechromatographed on silica gel, eluting with chloroform–methanol (96:4); recrystallization from methanol afforded compound **1** (80 mg). Fraction B was rechromatographed on silicagel (60–120 mesh), eluting with chloroform–methanol (90:10); recrystallization from methanol afforded compound **2** (1.5 g). The compounds visualization was done under UV light, also shown brown spot by spraying with 10% sulphuric acid in methanol.

Preparation of iridoid derivatives

General procedure for the dihydro compound

To a magnetically stirred solution of compound **2** (100 mg, 0.00018 mol) in methanol (10 ml) was added gradually $NiCl_2 \times 6H_2O$ (0.00009 mol) at rt. When the clear solution acquired a greenish colour, the whole reaction mixture was brought to 0 °C and $NaBH_4$ (0.00036 mol) was added portion wise. After addition of $NaBH_4$, the whole solution was stirred for 30 min at 0 °C to rt. Methanol was removed by vacuum, and then extracted with ethyl acetate (3 × 25 ml), the organic layer was washed with water, dried over anhyd Na_2SO_4 and evaporated under reduced pressure. Then the crude product was chromatographed on silica gel to afford the desired compound (Fig. 1).

General procedure for the amino hydroxylation

$K_2[OsO_2(OH)_2]$ (1.5 mol%) was dissolved with stirring in 10 ml of aqueous solution of $LiOH{\cdot}H_2O$. After the addition of *t*-BuOH, $(DHQ)_2PHAL$ was added and the mixture immersed in a cooling bath set 0 °C. After the addition of compound **2**, N-bromo acetamide was added in one portion, which resulted in an immediate colour change to green and the mixture vigorously stirred at the same temperature. The reaction was monitored by TLC, and pH (full conversion is indicated when the reaction mixture attains pH 7). After 4 h, the reaction mixture was treated with Na_2SO_3 and stirred at rt

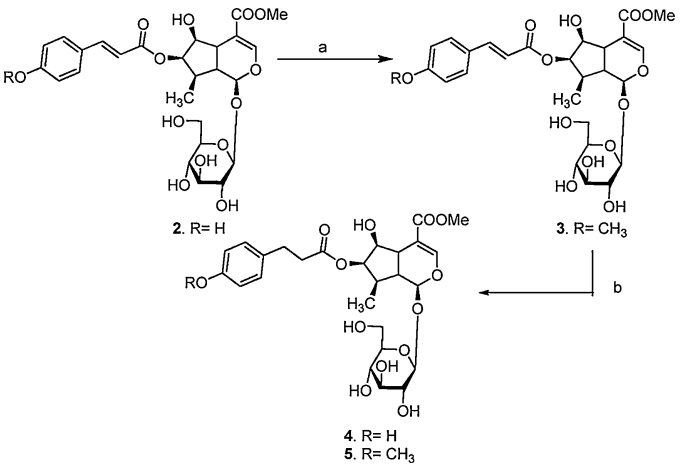

Fig. 1. Regioselective hydrogenation of compounds **2** and **3** by using $NaBH_4/NiCl_2{\cdot}6H_2O$.