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Anticancer activity and toxicity profiles of 2-benzylidene indanone lead molecule

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ABSTRACT

3-(3′,4′,5′-Trimethoxyphenyl)-4,5,6-trimethoxy,2-(3″,4″-methylenedioxybenzylidene)-indan-1-one (**1**) is an optimized anti-cancer lead molecule obtained on modification of gallic acid, a plant phenolic acid. It exhibited potent cytotoxicities (IC_{50} = 0.010–14.76 μM) against various human carcinoma cells. In cell cycle analysis, benzylidene indanone **1** induced G2/M phase arrest in both MCF-7 and MDA-MB-231 cells. It also induced apoptosis in DU145 cells which was evident by cleavage of PARP. In Ehrlich ascites carcinoma, benzylidene indanone **1** showed 45.48% inhibition of tumour growth at 20 mg/kg dose in Swiss albino mice. Further, in sub-acute toxicity experiment in Swiss-albino mice, it was found to be non-toxic up to 100 mg/kg dose for 28 days. The lead compound benzylidene indanone **1** can further be optimized for better anti-cancer activity.

1. Introduction

Cancer has become a major health problem. Over the years, the continuous increasing morbidity and mortality, have disturbed the policy makers a lot, which is about 13% of total human deaths at present (WHO Cancer factsheet 2015). Researchers are tirelessly exploring for a suitable anticancer drug without any side effect. Various types of approaches are being explored to tackle the disease.

Plants have been an excellent source of medicines for the treatment of various types of disorders as crude drug and as pure isolates as well (Cragg et al., 2014). It is estimated that natural products and their derived products contribute about 40% of total released drugs. Logical modification of natural products is well adopted approach by the researchers now. It is also evident that a particular fragment (motif) is much more crucial for receptor interaction and thus small molecule research has become important nowadays (Zartler, 2014; Negi et al., 2015).

Abbreviations: CSIR, Council of Scientific and Industrial Research; MTT, (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole; EAC, Ehrlich ascites carcinoma; ESI-MS, electrospray ionisation mass spectrometry; HRMS, high resolution mass spectrometry; DMEM, Dulbecco's Modified Essential Eagle Medium; FBS, Fetal Bovine Serum; PBS, Phosphate Buffer Saline; ER+, estrogen receptor positive; OECD, Organization for Economic Co-operation and Development; SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase; PARP, poly (ADP-ribose) polymerase.

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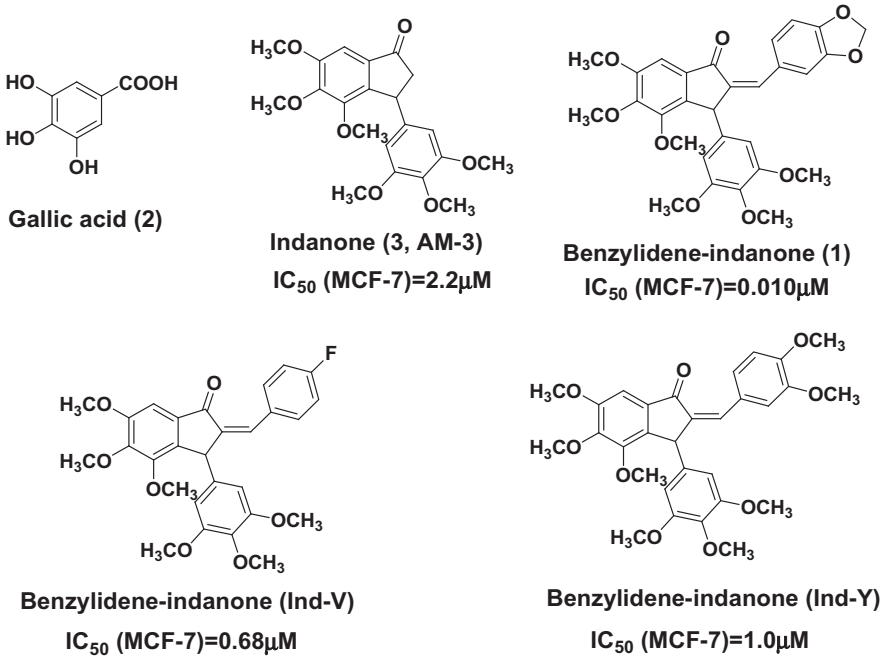

Fig. 1. Structures of gallic acid, indanone (AM-3) and three optimized benzylidene indanones (**1**, Ind-V and Ind-Y).

In our studies on gallic acid (**2**), a plant phenolic acid, previously, we designed few indanones as possible anticancer agents (Saxena et al., 2008) (Fig. 2). These indanones have been designed based on structural learnings from some of the natural antitubulins *viz.* colchicine, podophyllotoxin and combretastatin A4 etc. All these possess a 3,4,5-trimethoxyphenyl fragment which has affinity to bind with microtubules to induce tubulin polymerisation inhibition (Negi et al., 2015). This fragment binds with microtubule at colchicine binding site and induces antitubulin effect. In our design, a 3,4,5-trimethoxyphenyl motif was kept in the A ring of indanone pharmacophore to induce cytotoxicity through antitubulin effect.

We optimized an anticancer lead molecule i.e. 3-(3′,4′,5′-trimethoxyphenyl)-4,5,6-trimethoxyindanone-1 (**2, AM-3**) by synthesizing several 2-benzylidene indanones (Prakasham et al., 2012; Chanda et al., 2012; Negi et al., 2014) (Fig. 1). Three of these analogues exhibited potent anticancer activity (IC_{50} = 10–1000 nM) against MCF-7 hormone dependent breast cancer cell lines. In the present communication, we have explored the best analogue of the series i.e. benzylidene indanone **1** for cytotoxicity against few more human cancer cell lines, cell cycle analysis and apoptosis induction. Finally, benzylidene indanone **1** has also been evaluated for *in-vivo* anticancer activity and *in-vivo* sub-acute oral toxicity in Swiss-albino mice.

2. Materials and methods

2.1. General experimental procedures

The starting substrate gallic acid was procured from S.d. Fine Chemicals, India. Reagents and other chemicals were purchased either from Sigma–Aldrich, USA or Avra Chemicals India and used without purification. Melting points were determined in open glass capillaries on E-Z Melt automated melting point apparatus and were uncorrected. Reactions were monitored on Merck pre-coated silica gel TLC-GF_{254} aluminum sheets and compounds visualization was done under UV light (254 nm and 365 nm) and further charring with 2% ceric sulphate in 10% sulphuric acid (aqueous). Compounds were purified through Flash chromatography system (CombiFlash R_f200i, Teledyne-ISCO, USA) using glass columns (13 cm length × 2 cm i.d.) and silica gel (230–400 mesh) using UV detector (230 nm & 254 nm) and characterised by ^1H and ^{13}C NMR, ESI–MS, and ESI–HRMS. The purity of the final compound benzylidene indanone **1** was ascertained by Reversed Phase-HPLC. NMR spectra were obtained on Bruker Avance-300 MHz instrument with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in δ ppm values. ^1H–^1H coupling constant (*J*) values are given in Hz. ESI mass spectra were recorded on APC3000 LC–MS–MS (Applied Biosystem) and High Resolution Mass (HRMS) on Agilent 6520Q-TOF after dissolving the compounds in methanol. FT-IR spectra were recorded on Perkin–Elmer SpectrumBX. Purity profile of the investigating compound was done in Shimadzu LC–MS. Optical rotations were obtained using Horiba SEPA-300 (Japan) polarimeter at wavelength 5890 Å.

DMEM and FBS were purchased from Gibco, India. RNaseA, Crystal Violet Dye, HEPES, Trypsin-EDTA, Antibiotic–Antimycotic (Ab–Am) Solution, Phosphate Buffer Saline (PBS), Citric Acid and Propidium Iodide (PI) were acquired from Sigma–Aldrich, USA. Sodium bicarbonate ($NaHCO_3$), Agar, Sodium Citrate and Di-sodium Hydrogen Phosphate were obtained from Himedia Laboratories, India. Solvents including ethanol and isopropanol were procured from Merck, India Ltd. *In-vivo* experiments were conducted on Swiss-albino mice after the approval of Animal Ethical Committee.

2.2. Biological evaluation

MCF-7 (Human breast adenocarcinoma, ER+), MDA-MB-231 (Human breast adenocarcinoma, ER−) were originally obtained from American type of cell culture collection (ATCC) and grown at 37 °C in DMEM supplemented with 10% FBS and Ab–Am (antibiotic–antimitotic) solution in a CO_2 incubator (New Brunswick/Eppendorff, Germany) under 5% CO_2 and 95% relative humidity.