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Synthesis, characterization and biological evaluation of bile acid-aromatic/heteroaromatic amides linked *via* amino acids as anti-cancer agents

Devesh S. Agarwal [a], Hasitha Shilpa Anantaraju [b], Dharmarajan Sriram [b], Perumal Yogeeswari [b], Shankara H. Nanjegowda [c], P. Mallu [c], Rajeev Sakhuja [a,*]

[a] Department of Chemistry, Birla Institute of Technology and Science, Pilani 333 031, Rajasthan, India
[b] Drug Discovery Research Laboratory, Department of Pharmacy, Birla Institute of Technology & Science-Pilani, Hyderabad Campus, Hyderabad 500078, India
[c] Department of Chemistry, Sri Jayachamarajendra College of Engineering, Mysore 570006, India

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ABSTRACT

A series of bile acid (Cholic acid and Deoxycholic acid) aryl/heteroaryl amides linked *via* α-amino acid were synthesized and tested against 3 human cancer cell-lines (HT29, MDAMB231, U87MG) and 1 human normal cell line (HEK293T). Some of the conjugates showed promising results to be new anticancer agents with good *in vitro* results. More specifically, Cholic acid derivatives **6a** (1.35 μM), **6c** (1.41 μM) and **6m** (4.52 μM) possessing phenyl, benzothiazole and 4-methylphenyl groups showed fairly good activity against the breast cancer cell line with respect to Cisplatin (7.21 μM) and comparable with respect to Doxorubicin (1 μM), while **6e** (2.49 μM), **6i** (2.46 μM) and **6m** (1.62 μM) showed better activity against glioblastoma cancer cell line with respect to both Cisplatin (2.60 μM) and Doxorubicin (3.78 μM) drugs used as standards. Greater than 65% of the compounds were found to be safer on human normal cell line.

1. Introduction

Primary bile acids such as Cholic acid (CA) and Chenodeoxycholic acid (CDCA) are endogenous steroids that are excreted into the bile canaliculus and the digestive tract after cholesterol catabolism [1]. Bacterial metabolism of tauryl and glycyl conjugates of CA and CDCA in distal small intestine and colon produces secondary bile acids such as Deoxycholic acid (DCA), Ursodeoxycholic acid (UDCA), and Lithocholic acid (LCA) [2]. Slight differences in the chemical structure of these bile acids make them behave distinctly in the biological environment. Earlier reports on hydrophobic bile acids indicated their cytotoxic behavior towards normal tissues [3], while less hydrophobic bile acid such as UDCA protect normal cells against apoptosis induced by direct prevention of mitochondrial membrane perturbation [4]. UDCA and its derivatives have been extensively studied as potent scaffolds in medicinal chemistry [5]. *In vitro* and animal studies have suggested the chemopreventive behavior of UDCA towards colorectal cancer [6]. UDCA has been used for the prevention of gastrointestinal disorders in patients having various cancers such as stomach, colon, lung, breast, and liver cancer [7]. UDCA itself has been evaluated as a drug in clinical phase III trial therapy to prevent colorectal adenoma recurrence [8]. Recently, Qui and co-workers reported anticancer effect of UDCA in human oral squamous carcinoma HSC-3 cells through the caspases [9]. UDCA and its metal complexes decreased the viability and proliferation of cultured human and animal tumor cells in a time- and concentration-dependent manner [10]. In addition, UDCA expresses an inhibitory effect on the induction of P-glycoprotein expression and reactive oxygen species by Doxorubicin in HepG2 human hepatoma cells [11].

In 2001, Powell and co-workers reported a direct correlation between bile acids hydrophobicity and induction of apoptosis and/or growth arrest in HCT116 cells, however no apparent link was observed between a particular structural modification and its biological activity [12]. Nonetheless, reports in the last two decades suggested the induction of apoptosis by hydrophobic bile acid derivatives in many human cancer cells [13], such as prostate cancer cells [14], leukemic T cells [15], hepatocellular carcinoma cells [16], colon cancer cells [17], breast carcinoma cells [18], osteosarcoma cells [19], and cervical carcinoma cells [20]. However, low cytotoxicity (IC_{50} > 100 μM) and tumor promoter ability of certain bile acid derivatives has compelled medicinal chemist to design more efficient bile acid derivatives with potent anti-proliferative

* Corresponding author.
E-mail address: sakhuja.rajeev@gmail.com (R. Sakhuja).

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and apoptotic properties. Thus, physiological and carcinogenesis studies of bile acid derivatives have experienced a significant progress in the last decade.

Many bile acid-amino acid conjugates have been studied for their anti-cancer properties. Among these, compounds HS-1183, HS-1199 and HS-1200 (Fig. 1) showed promising results in inducing apoptosis with an IC_{50} values from 25 to 50 μM for SiHa human cervical carcinoma cells, 30 to 45 μM for MDA-MB-231 cells and 30 to 150 μM for MCF-7. Reports also suggests unnatural long chain amino acid conjugates of bile acid to possess strong anti-cancer activity against several tumor cell lines including human breast adenocarcinoma (ER-, MDA-MB-231), breast adenocarcinoma (ER +, MCF-7), cervix epiteloid carcinoma, (HeLa S-3) and prostate cancer (PC-3) [21]. Khiel and co-workers reported piperazinyl linked aryl and heteroaryl substituted bile acid derivatives with apoptosis-inducing activity on multiple myeloma cancer cell line KMS-11 [22]. Bile acid–polyaminocarboxylate conjugates containing NE3TA, a potential iron chelator displayed significant cytotoxicity in both HeLa and HT29 colon cancer cells [23]. Other reports on the synthesis and structural studies of bile acid-4-aminopyridine conjugates [24], *N*-(2-aminoethyl)amido linked bile-acid–heteroaryl conjugates [25], bile acid-arene conjugates [26] and bile acid-glutamyl-pyridines [27] have been described, however no anticancer-activity evaluation studies were reported.

In view of the preceding discussion, we herein describe the design (Fig. 1) and synthesis of new bile acid-aromatic/heteroaromatic amides linked *via* amino acids. *In vitro* anti-cancer studies were accomplished on human breast adenocarcinoma cell line (MDA-MB-231), human colorectal adenocarcinoma cell line (HT29) and human glioblastoma cell line (U87MG).

2. Experimental

2.1. General

All the chemicals were purchased from Sigma-Aldich, Alfa Aesar, and Spectrochem India Pvt. Ltd and used without further purification. The solvents used were purchased from Merck (India) and were distilled and dried before use. Nuclear magnetic resonance spectra were recorded on Bruker 400 spectrometer. The ^1H NMR experiments were reported in δ units, parts per million (ppm), and were measured relative to residual chloroform (7.26 ppm) or DMSO (2.5 ppm) in the deuterated solvent. The ^{13}C NMR spectra were reported in ppm relative to deuterochloroform (77.0 ppm) or DMSO-d_6 (39.5 ppm). All coupling constants J were reported in Hz. The following abbreviations were used to describe peak splitting patterns when appropriate: s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet and br s = broad singlet. Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. Reactions were monitored by using thin layer chromatography (TLC) on 0.2 mm silica gel F254 plates (Merck). The chemical structures of final products were confirmed by a high-resolution ESI/APCI-hybrid quadrupole time-of-flight mass spectrometer. High resolution mass spectrometry (HRMS) was performed with a waters synapt G2 HDMS instrument using time-of-flight (TOF-MS) with ESI/APCI-hybrid quadrupole. Optical rotations were recorded using a Perkin Elmer 343 series polarimeter in methanol.

2.2. General synthesis of Boc protected aminoacyl aromatic/heteroaromatic amides

To the stirred solution of **1** (1 mmol) in DMF (20 mL), triethyl amine (2.5 mmol) was added at 0 °C and subsequently EDC.HCl (1.5 mmol) and HOBt (1 mmol) was added. The reaction mixture was stirred for 15 min. at 0 °C, after that aryl/heteroaryl amines (**2**) (1.1 mmol) was added and the reaction was stirred at room temperature for 6–8 h. The completion of the reaction was monitored by TLC. After the completion of the reaction, crushed ice was added. The resulted precipitate was filtered, washed with cold water and recrystallized with ethyl acetate/hexanes to yield pure **3a–g**.

2.2.1. tert-Butyl ((2S,3R)-3-methyl-1-oxo-1-(phenylamino)pentan-2-yl)carbamate (*3a*)

Off-white solid; yield: 81%, mp: 130–132 °C, ^1H NMR (400 MHz, $CDCl_3$) δ 8.56 (br s, 1H, NH_{Ar}), 7.47 (d, J = 8.0 Hz, 2H, H-3_{Ar} & H-5_{Ar}), 7.21 (t, J = 7.0 Hz, 2H, H-2_{Ar} & H-6_{Ar}), 7.03 (t, J = 7.2 Hz, 1H, H-4_{Ar}), 5.39 (d, J = 8.6 Hz, 1H, $NH_{isoleucine}$), 4.13 (t, J = 8.0 Hz, 1H, $CH_{isoleucine}$), 1.90–1.99 (m, 1H), 1.42 (s, 9H, 3 × Me_{Boc}), 1.30–1.07 (m, 2H), 0.99 (d, J = 6.8 Hz, 3H), 0.90 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.54 (C=O_{amide}), 156.30 (C=O_{Boc}), 137.63 (C-1_{Ar}), 128.79 (C-3_{Ar} & C-5_{Ar}), 124.20 (C-4_{Ar}), 119.95 (C-2_{Ar} & C-6_{Ar}), 80.16, 60.07 (NH—**C**$H_{isoleucine}$), 37.00, 28.30, 24.89, 15.57,

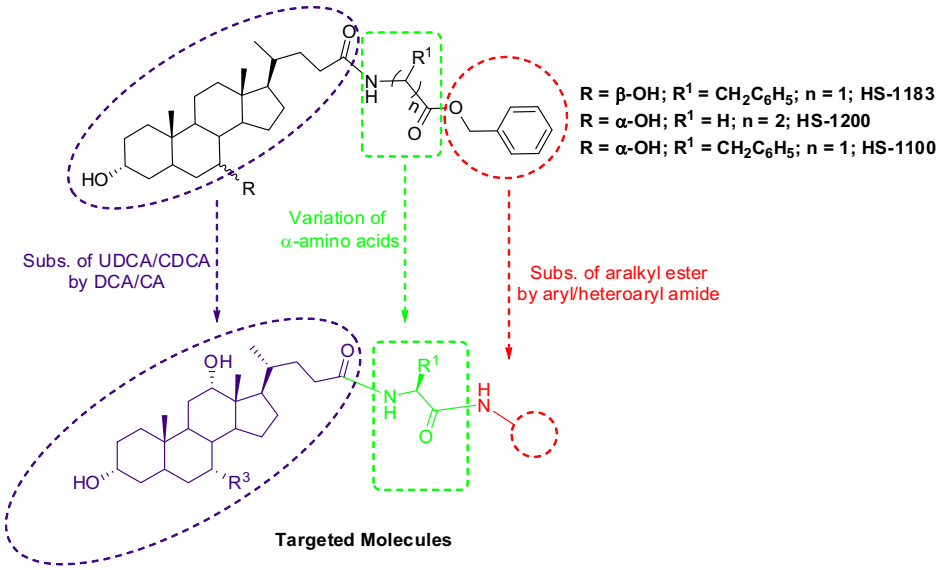

Fig. 1. Design of targeted molecules with reference to known anti-cancer bile acid-amino acid conjugates.