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Original article

Synthesis, screening and pro-apoptotic activity of novel acyl spermidine derivatives on human cancer cell lines

Syed Shoeb Razvi[a], Hani Choudhry[a,b,c,e], Said Salama Moselhy[a,d,e,f], Taha Abduallah Kumosani[a,d,g], Mohammed Nihal Hasan[a], Mazin A. Zamzami[a,b,c], Khalid Omer Abualnaja[a,d,e], Abdulrahman Labeed Al-Malki[a,d,e], Mahmoud Alhosin[a,b,c,1,**], Tadao Asami[a,h,1,*]

[a] Department of Biochemistry, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[b] Cancer Metabolism and Epigenetic Unit, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia
[c] Cancer and Mutagenesis Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[d] Experimental Biochemistry Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia
[e] Bioactive Natural Products Research Group, King Abdulaziz University, Jeddah, Saudi Arabia
[f] Biochemistry Department, Faculty of Science, Ain shams University, Cairo, Egypt
[g] Production of Bioproducts for Industrial Applications Research Group, King Abdulaziz University, Jeddah, Saudi Arabia
[h] Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo, 113-8657, Japan

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ABSTRACT

The polyamines putrescine, spermidine, and spermine are polycationic, alkyl polyamines which play a significant role in eukaryotic cell proliferation. The polyamine metabolism and function are dysregulated in tumor cells making them an attractive therapeutic target by employing polyamine analogs. These analogs have a high degree of similarity with the structure of polyamines but not with their function. Multidrug resistance is a major factor in the failure of many chemotherapeutic drugs which necessitates further research and exploration of better novel alternatives. In the present study, Twenty-six novel acylspermidine derivatives were synthesized and evaluated for their anti-proliferative and pro-apoptotic activities on human breast cancer cells and T-lymphoblastic leukemia cells. The cell proliferation and apoptosis assays using WST-1 and annexin-V/7AAD staining respectively suggest that Compound **1** ($C_{19}H_{41}N_3O_2$), Compound **7**($C_{25}H_{51}N_3O_2$) and Compound **8** ($C_{29}H_{59}N_3O$) significantly reduced cancer cell viability in a dose- and time-dependent manner. Interestingly, compounds **7, 8** and **9** had slight or no effect on cell proliferation of non-cancerous cells. These studies speculate that these novel acylspermidine derivatives could be promising candidates in designing an anti-proliferative drug, targeting both solid and blood cancer cells.

1. Introduction

Cancer still remains the most common cause of death worldwide with the gradual increase of the incidences with age. Though initially, it responds to the chemotherapy or the radiation therapy but with the due course of time and treatment, cells acquire resistance through mutation or downregulation of the pro-apoptotic proteins and the overexpression of anti-apoptotic proteins such as bcl-2 [1,2]. Carcinogenesis involves dysregulation of the complex processes like apoptosis, angiogenesis and cell proliferation. Usage of phytochemicals or the synthetic analogs of the metabolites involved in the cell proliferation is on the rise for the treatment of the cancerous lesions triggering apoptosis. Many anticancer chemotherapeutic drugs have been developed which induce apoptosis [3,4].

Polyamines are positively charged molecules which have a crucial role in the proliferation and normal growth of a cell [5]. They are known for their unique interaction with a variety of biological targets, mostly with membrane phospholipids and

* Corresponding author at: Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo, Tokyo, 113-8657, Japan.
** Corresponding author at: Department of Biochemistry, Cancer Metabolism and Epigenetic Unit, Faculty of Sciences, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia.
E-mail addresses: malhaseen@kau.edu.sa (M. Alhosin), brassinazole@gmail.com (T. Asami).
[1] Equal contributors.

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nucleic acids through the electrostatic interactions completing the protonation of amino groups at physiological pH [6]. Spermine, spermidine, and putrescine are the natural polyamines studied widely due to the various receptors, ion channels, and the other recognition sites to which they bind [7]. Many ligands have been designed with different and specific biological targets by insertion of suitable pharmacophores onto the backbone of polymethylene including the adjustment of the position of the amine which can optimize the selectivity and affinity of the receptor [2,5,8].

During the recent years, many compounds of polyamine backbone have been synthesized which demonstrated anti-proliferative effect against diverse malignant tumors. Synthetic polyamine analogs like bisnaphthalimido or N1, N11-diethyl norspermine against breast cancer are significant with regard to anti-neoplastic activities against many solid tumor models of humans [9,10].

Many of the anticancer drugs that are widely clinically used today have high risks of adverse side effects and poor specificity and selectivity against tumors. Hence, there arises an urgent need to encounter this problem by improving the efficacy, selectiveness, and safety of the anti-tumor agents which can be done by modification with specific polyamine motifs [7] elevating the affinity for tumor cells and reach the biological targets. Therefore, we synthesized novel conjugates of acyl polyamines with fatty acyl group as a primary polyamine motif on the polyamine backbone. It has been hypothesized by many researchers that breast cancer is one of the most chemo-sensitive solid tumors, most of the initially responsive tumors regress and then develop resistance to a broad spectrum of drugs [11,12].

Moreover, the human breast cancer cell line MCF-7 is considered as the most common malignant type of breast cancer used widely around the laboratories as a tumor model in screening to study the anticancer effects and also in the investigation of the mechanism of action of hormones [13–15]. Additionally, the T-lymphoblastic leukemia cell line Jurkat is also a documented experimental model for hematological cancers in the screening of the anticancer activity as well as the cellular and molecular mechanisms of drugs exhibiting anticancer proprieties [16–18].

The aim of this study was to evaluate the anti-proliferative and pro-apoptotic effects of synthetic acylspermidines on the human adenocarcinoma cell line MCF-7 and the T-lymphoblastic leukemia cell line Jurkat.

2. Material and methods

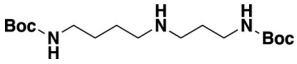
2.1. The general scheme of synthesis of active compounds

The general scheme of synthesis of the compounds is depicted in Fig. 1.

2.2. Synthesis of N^4-(3-hydroxylauroyl) spermidine (compound 1)

2.2.1. N^1,N^8-bis-tert-butoxycarbonylspermidine

2-(*tert*-Butoxycarbonyloxyimino)-2-phenylacetonitrile (4.85 g, 19.7 mmol) in dry THF (25 mL) was added dropwise to a solution of spermidine (1.36 g, 9.36 mmol) and triethylamine (3.91 mL, 28.1 mmol) in dry THF (25 mL) with cooling in an ice bath and then stirred overnight at room temperature. After the reaction, the solvent was removed in vacuo, and 1 M NaOH (50 mL) was added to the resultant residue. The aqueous layers were extracted with dichloromethane (50 mL × 3). The combined organic layers were washed with saturated aqueous sodium chloride (50 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give white solid, which was then recrystallized with *n*-hexane/dichloromethane to give white solid of N^1,N^8-bis-*tert*-butoxycarbonylspermidine (2.58 g, 7.47 mmol, 79.8%).



1H NMR (500 MHz, $CDCl_3$) δ 5.13 (s, 1H, NHCO), 4.80 (s, 1H, NHCO), 3.27–3.15 (m, 2H, $C\underline{H}_2NHCO$), 3.14–3.02 (m, 2H, $C\underline{H}_2NHCO$), 2.65 (t, J = 6.6 Hz, 2H, $(C\underline{H}_2)_4NHC\underline{H}_2$), 2.59 (t, J = 6.6 Hz, 2H, $(CH_2)_3NHC\underline{H}_2$), 1.72–1.58 (m, 2H, $NHCH_2C\underline{H}_2CH_2NHCO$), 1.58–1.46 (m, 4H, $NHCH_2C\underline{H}_2C\underline{H}_2CH_2NHCO$), 1.42 (s, 18H, $C(CH_3)_3$).

2.2.2. N^1, N^8-bis-tert-butoxycarbonyl-N^4-(3-hydroxylauroyl) spermidines

Under nitrogen atmosphere, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (665 mg, 3.47 mmol) was added to a solution of N^1, N^8-bis-*tert*-butoxycarbonylspermidine (1.00 g, 2.89 mmol), 3-hydroxylauric acid (626 mg, 2.89 mmol) and dimethylamino pyridine (424 mg, 3.47 mmol) in dichloromethane (20 mL), and stirred for 2 days at room temperature. Then a solution of 10% (w/w) citric acid (10 mL) was added to the reaction

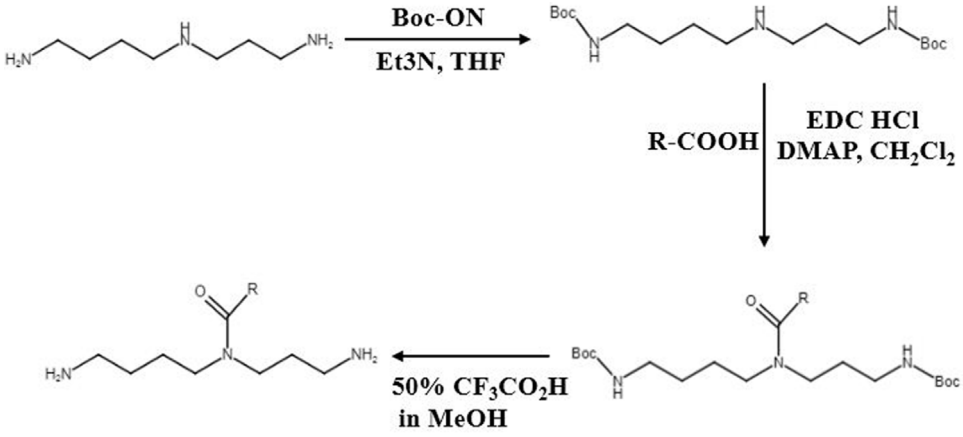


Fig 1. Overview of synthesis of active compounds. Compound **1**: (R = -CH_2-CH(OH)-$(CH_2)_7$-CH_3); Compound **7**: (R = -CH_2-$(CH_2)_5$-CH_2-CH = CH-CH_2-CH(OH)-$(CH_2)_5$-CH_3); Compound **8**: (R = -CH_2-$(CH_2)_9$-CH_2-CH = CH-CH_2-$(CH_2)_7$-CH_3); Compound **9**:(R = -CH_2-$(CH_2)_6$-CH_2-CH = CH-CH_3).