Bioorganic Chemistry 65 (2016) 73–81

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis and antiproliferative activity of benzophenone tagged pyridine analogues towards activation of caspase activated DNase mediated nuclear fragmentation in Dalton's lymphoma

Mohammed Al-Ghorbani [a], Prabhu Thirusangu [b], H.D. Gurupadaswamy [a], V. Girish [a], H.G. Shamanth Neralagundi [b], B.T. Prabhakar [b], Shaukath Ara Khanum [a,*]

[a] Department of Chemistry, Yuvaraja's College, University of Mysore, Mysore 570005, Karnataka, India
[b] Molecular Biomedicine Laboratory, Postgraduate Department of Studies and Research in Biotechnology, Sahyadri Science College (A), Kuvempu University, Shimoga 577203, Karnataka, India

ARTICLE INFO

Article history:
Received 1 January 2016
Revised 1 February 2016
Accepted 2 February 2016
Available online 2 February 2016

Keywords:
Benzophenone-pyridine
DLA
CAD
Endonuclease

ABSTRACT

A series of benzophenones possessing pyridine nucleus **8a–l** were synthesized by multistep reaction sequence and evaluated for antiproliferative activity against DLA cells by *in vitro* and *in vivo* studies. The results suggested that, compounds **8b** with fluoro group and **8e** with chloro substituent at the benzoyl ring of benzophenone scaffold as well as pyridine ring with hydroxy group exhibited significant activity. Further investigation in mouse model suggests that compounds **8b** and **8e** have the potency to activate caspase activated DNase (endonuclease) which is responsible for DNA fragmentation, a primary hallmark of apoptosis and thereby inhibits the Dalton's lymphoma ascites tumour growth.

1. Introduction

Programmed cell death (PCD) or apoptosis, plays a very crucial role in the maintenance of tissue homeostasis by regulating cell death [1]. When there is a failure in the cell death, it leads to the malignant tumour [2,3]. During apoptosis, cells exhibit specific morphological changes. These include membrane blebbing, cytoplasmic condensation, chromatin condensation, nucleosomal fragmentation and apoptotic body formation [4]. The fragmentation of chromosomal DNA into nucleosomal units, producing small DNA fragments or DNA ladder, is considered as a prominent biochemical hallmark of apoptotic cell death [4,5]. The molecular characterization of this process identifies a specific DNase called Caspase activated DNase (CAD) or DNA fragmentation factor (DFF-40) that cleaves cellular DNA in a caspase-dependent manner that eradicates the cells in an appropriate way [6,7]. Apoptosis can be activated through awakening CAD by dissociating the inhibitor of CAD (ICAD) from CAD which normally heterodimerised with ICAD [8–10]. In this manner, the development of the active, selective and less toxic candidates, which targets the activation of CAD has become a key strategy for cancer treatment.

Pyridine is one of the most prevalent heterocyclic compounds in nature. For example, it is present in the coenzyme vitamin B_6 family and in numerous alkaloids, further it plays a central role as versatile building block in the synthesis of natural products as well as biologically active compounds. Further, pyridine bases are widely used in pharmaceutics as nicotinamides and nicotinic acid derivatives. The various therapeutic potential of pyridine derivatives have been reported in the treatment of cancers of diverse cells, by targeting angiogenesis [11,12], apoptosis [13,14] and by inhibiting wide range of tumour promoting factors like, FAK [14], CDK [13,16] and topoisomerase II [17]. Nevertheless, benzophenone derivatives are extensively used in medicine research for their recognized potencies against various pathological conditions including cancer [18–20]. In recent years, our group has reported a number of novel benzophenone conjugated analogues as potent inhibitors targeting angiogenesis [21–23] and apoptosis [24,25]. In continuation, this current study is based on the synthesis of benzophenone bearing pyridine nucleus and their evaluation for antiproliferative and apoptogenic properties against Dalton's lymphoma by both *in vitro* and *in vivo* analysis.

* Corresponding author.
E-mail address: shaukathara@yahoo.co.in (S.A. Khanum).

http://dx.doi.org/10.1016/j.bioorg.2016.02.001
0045-2068/

2. Results and discussion

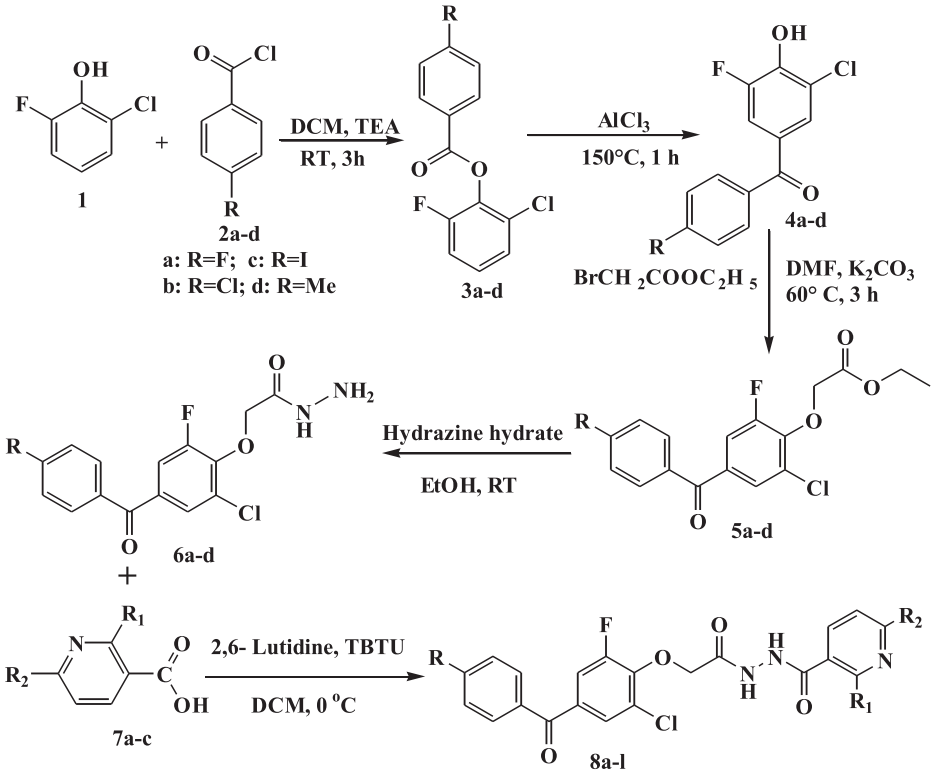
2.1. Chemistry

The reaction sequence for various title compounds **8a–l** was outlined in Scheme 1. Substituted phenyl benzoates **3a–d** were synthesized by stirring 2-chloro-6-fluoro phenol **1** with substituted acid chlorides **2a–d** in alkaline medium using triethylamine. The phenyl benzoates **3a–d** were subjected to Fries rearrangement to afford hydroxy benzophenones **4a–d.** Condensation of **4a–d** with ethyl chloroacetate in the presence of anhydrous potassium carbonate in dry acetone gave ethyl (2-aroyl-4-methylphenoxy) acetates **5a–d**, which on treatment with 99% hydrazine hydrate in ethanol gave 4-aryloylaryloxyacethydrazides **6a–d**. Finally, the title compounds **8a–l** were achieved in excellence yield by coupling **6a–d** with substituted nicotinic acids **7a–c** in the presence of 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as coupling agent and 2,6-dimethyl pyridine (lutidine). The structures of the compounds were elucidated by IR, ^{1}H NMR and mass spectral studies and also by microanalyses.

2.2. Biology

2.2.1. In vitro selection of the lead compound and their structure activity relationships

During the last decade, the researchers focused on benzophenone derivative and suggest that the benzophenone conjugated with various specific bioactive molecules such as benzimidazole [21], coumarin [22], thiazole [23], oxadiazole [24] and acetamide [25,26] has a promising pharmacological activity against various pathological conditions including cancer. While, pyridine or nicotinic acid is well known for its biological activity against carcinogenesis [14–19]. In the present investigation, compounds **8a–l** analogues were synthesized, by tagging the benzophenone with pyridine ring and evaluated for their antiproliferative and cytotoxic properties against Dalton's Lymphoma Ascites (DLA) cells by performing MTT, trypan blue and lactate dehydrogenase (LDH) release assays [Fig. 1]. In the series of compounds **8a–l,** the compounds **8b** with fluoro and **8e** with chloro group at the para position in the benzoyl ring of the benzophenone and also with hydroxy pyridine moiety exhibited a noticeable cytotoxic effect against DLA cells. The compounds **8b** and **8e** were found to be exhibiting a promising antiproliferative effect with IC_{50} of 8.5 μM and 9.3 μM respectively in MTT assay [Fig. 1A]. The synchronized results were obtained for both the compounds **8b** and **8e** in trypan blue dye exclusion assay with the cytotoxic effects at IC_{50} of 8.7 μM and 9.5 μM respectively [Fig. 1B]. The cellular integrity of the title compounds was evaluated using the LDH release assay [Fig. 1C], which is one of the gold standard methods to assess the cytotoxic effect of the compounds. The results obtained from LDH release assay showed that there was an increase in the release of LDH after the treatment with compounds **8b** and **8e,** which exhibited IC_{50} of 8.5 μM and 9.4 μM respectively. In the direction of structure and structure activity relationship (SAR), the compounds **8h** and **8k** with hydroxy pyridine moiety, but different substituents at the para position in the benzoyl ring of benzophenone exhibited moderate antiproliferative activity whereas, compounds **8b** and **8e** exhibited noticeable antiproliferative activity. Surprisingly, the compounds



Scheme 1. Synthesis of nicotinic acid N′-[2-(4-benzoyl-phenoxy)-acetyl]-hydrazides **8a–l**.