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Research paper

Nitric oxide-releasing derivatives of brefeldin A as potent and highly selective anticancer agents

Kangtao Tian [a, 1], Fanxing Xu [b, 1], Xiang Gao [a], Tong Han [a], Jia Li [a], Huaqi Pan [c, **], Linghe Zang [d], Dahong Li [a, ***], Zhanlin Li [a], Takahiro Uchita [e], Ming Gao [e], Huiming Hua [a, *]

[a] Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, and School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China
[b] Wuya College of Innovation, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China
[c] Shenyang Institute of Applied Ecology, Chinese Academy of Sciences, 72 Wenhua Road, Shenyang 110016, PR China
[d] School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China
[e] School of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien, Nishinomiya 663-8179, Japan

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ABSTRACT

A series of NO-donating mono- or diester derivatives of brefeldin A were designed, synthesized and biologically evaluated. Some derivatives exhibited potent antiproliferative activity with low IC_{50} values. The most potent NO-donating hybrid **13b** exhibited stronger cytotoxicity against human prostate cancer PC-3 cells, human colon carcinoma HT-29 cells and human liver cancer HepG-2 cells than BFA with IC_{50} values of 25 nM, 160 nM and 180 nM, respectively. More importantly, compound **13b** showed good selectivity between human normal and tumor liver cells with selectivity index of 33. Additionally, **13b** released higher levels of NO in HepG-2 cells than L-02 cells. Further mechanism concerning cellular apoptosis showed that **13b** induced apoptosis and S phase cell cycle arrest in HepG-2 cells. Incubation with **13b** increased the number of HepG-2 cells with collapsed mitochondrial membrane at low concentrations in dose-dependent manner. In addition, by using the Human Apoptosis Protein Array kit, several apoptosis-related proteins, including HO-1, HO-2 and survivin, were found to be markedly downregulated by **13b** in HepG-2 cells. Furthermore, in western blot assay, **13b** increased the expression of Bax, Cyt *c* and caspase 3, and reduced the relative levels of Bcl-2, Bcl-xl and pro-caspase 3 in HepG-2 cells.

1. Introduction

Brefeldin A (BFA) (**1**, Fig. 1) was first isolated from *Penicillium decumbens* by Singleton et al. [1]. It is a macrolide antibiotic and secondary metabolite of *Ascomycetes* species. Most importantly, BFA exhibited a variety of biological activities including antitumor [2], antifungal [3], antiviral [4], antimitotic [5] and so on. The potent antiproliferative activity of BFA was tested by the National Cancer Institute's 60 cancer cell line assay (NCI-60) with the mean graph midpoint (MGM) GI_{50} value of 40 nM [6]. Furthermore, the mechanism was widely investigated, that BFA could induce apoptosis in numerous human cancer cell lines [7,8] and disrupt the *cis* Golgi apparatus [9]. Treatment of eukaryotic cells with BFA could induce the breakdown of vesicle-mediated protein transport, which resulted in the Golgi complex redistributing into the endoplasmic reticulum (ER) [10]. Moreover, it has been reported that BFA would cause apoptotic cell death in ovarian carcinoma cell lines by activating the mitochondrial pathway and the caspase-8- and Bid-dependent pathways [11]. In addition, BFA also effectively inhibited clonogenic activity and the migration and matrix metalloproteinases-9 activity of MDA-MB-231 cells. Western blot analysis indicated that BFA could mediate the down-regulation of breast cancer stem cells marker CD 44 and anti-apoptotic proteins Bcl-2 and Mcl-1, as well as the reversal of epithelial-mesenchymal transition [12]. These biological results and mechanism investigation prompted BFA to be a potential therapeutic lead for future investigation [13,14].

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: panhq@iae.ac.cn (H. Pan), lidahong0203@163.com (D. Li), huimhua@163.com (H. Hua).
[1] These authors contributed equally to this work.

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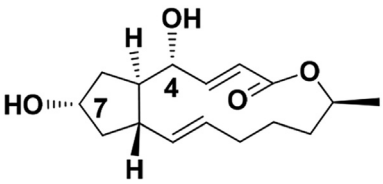


Brefeldin A (BFA), 1

Fig. 1. Chemical structure and key numbering of brefeldin A.

Although the cytostatic and apoptotic effects of BFA have been well studied, it is unsuitable to subject BFA to direct clinical development mainly because of its undesirable drug-like properties [15,16], including low selectivity between tumor and normal cells. In this study, the antiproliferative activity, especially the selectivity between cancerous and normal cells was improved, which was probably contributed to the introducing of nitric oxide (NO) donor moieties.

NO is known as a notable mediator in biological system and a wide range of physiological processes [17,18]. Active macrophages produce NO, which induce cytostasis and generate cytotoxity on tumor cells, and the mechanism seems to be the inhibition of ribonucleotide reductase [19]. Recently, NO donor hybrid compounds have become a focus in the field of oncotherapy since NO influences the apoptosis process of tumor cells [18]. It is reported that OA/furoxan hybrid compounds exhibited remarkably improved cytotoxicity because of the cytotoxic effects of NO released from the furoxan moieties [20]. In addition, nonsteroidal anti-inflammatory drugs/nitrate hybrids have been identified as the prototypical chemopreventive agents against many forms of cancers [21]. We have also synthesized a variety of furoxan-based NO donating compounds, such as evodiamine/furoxan [22] and spirolactone-type diterpenoid/furoxan hybrids [23] with potent antitumor activities and/or selectivity between cancerous and normal cells. Therefore, generation of furoxan-based hybrids is a promising strategy to develop potential antitumor drug candidates.

On the basis of above, the present article outlined the synthesis of 18 mono- or diester derivatives of BFA, which were obtained by introducing furoxan-type NO donors into the two hydroxyl (at C4 and C7 position) groups. The cytotoxicity, NO releasing ability and cellular mechanism of these derivatives were investigated. The research herein aimed to obtain a befitting dual BFA/NO releasing agent that still maintained the antiproliferative activity and showed selectivity between cancerous and normal cells.

2. Results and discussion

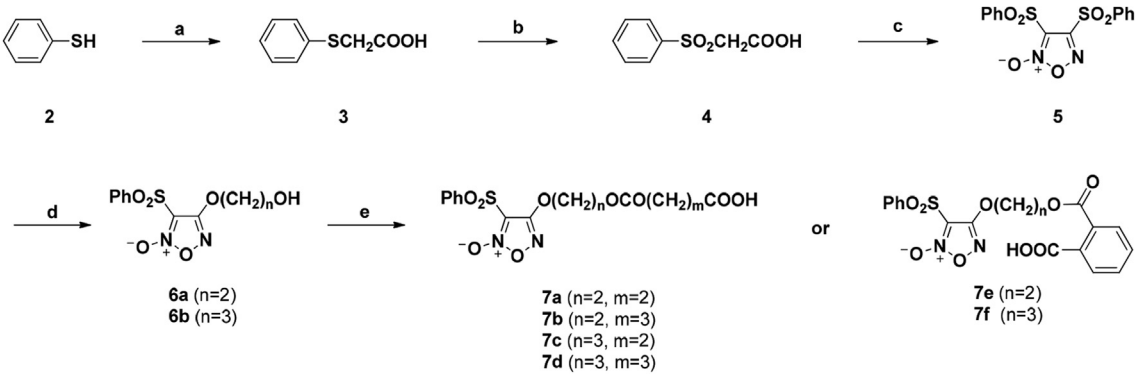
2.1. Chemistry

The derivatives **11a**–**f**, **12a**–**f** and **13a**–**f** were synthesized from thiophenol and BFA. The BFA was isolated from the fermentation liquor and mycelium of *Eupenicillium brefeldianum*, and characterized by ^{1}H NMR, ^{13}C NMR, high-resolution mass spectra, X-ray crystal structure analysis and optical rotation [24]. Thiophenol **2** was treated with chloroacetic acid in sodium hydroxide yielding the (phenylthio)acetic acid **3**. A one-pot reaction of (phenylthio) acetic acid by oxidization with 30% H_2O_2 produced compound **4**, which was further reacted with fuming HNO_3 at 90 °C to yield **5**. Reacting **5** with ethanediol or propanediol in the presence of 30% NaOH in THF afforded **6a**–**b**, which were further treated with anhydride, triethylamine (Et_3N) and 4-dimethylaminopyridine (DMAP) to obtain intermediates **7a**–**f** (Scheme 1). In order to get the ester derivatives at 4-position, the 4,7-OH groups of BFA were protected with *tert*-butyldimethylsilyl (TBS) groups using *tert*-butyldimethylsilyltriflate (TBSOTf) and 2,6-lutidine in dichloromethane (DCM). Then the TBS group on 4-OH of compound **8** was removed by *tert*-butylammonium fluoride (TBAF) in THF to obtain the intermediate **9**. Reacting compound **9** with intermediates **7a**–**f** in the presence of DMAP and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) afforded the intermediates **10a**–**f**. The derivatives **11a**–**f** were got from **10a**–**f** by the reaction with TBAF in THF (Scheme 2). Target compounds **12a**–**f** and **13a**–**f** were prepared from the combination of **7a**–**f** and BFA under the condition of DMAP and EDCI in DCM, followed by column chromatography on silica gel using petroleum ether-ethyl acetate (V:V 2:1) (Scheme 3). Intermediate **7a** was further treated with $TMSCHN_2$ to offer compound **14**. **15** was prepared by the reaction of BFA with succinic anhydride in 2,6-lutidine (Scheme 4).

2.2. Pharmacology

2.2.1. The antiproliferative activities of BFA derivatives

Applying the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay, the antiproliferative activities of BFA and the derivatives against one normal (L-02) and three tumor (PC-3, HT-29 and HepG-2) cell lines were evaluated and the results were summarized in Table 1. 5-FU was served as positive control. Comparing the antiproliferative activities of NO-donating BFA derivatives (**11a**–**f**, **12a**–**f** and **13a**–**f**) with those of NO donors **7a**–**f** and BFA derivative **15**, it could be found that the hybrids showed better antiproliferative profiles. It



Scheme 1. Synthesis of NO donors **7a**–**f**. Reagents and conditions: (a) $ClCH_2COOH$, NaOH (aq), 140 °C, 2 h; (b) 30% H_2O_2, AcOH, rt, 3 h; (c) fuming HNO_3, AcOH, 90 °C, 4 h; (d) (1) $HOCH_2CH_2OH$, THF, 30% NaOH, 0 °C, 4–8 h; (2) $HOCH_2CH_2CH_2OH$, THF, 30% NaOH, 0 °C, 72 h; (e) (1) succinic anhydride, Et_3N, DMAP, DCM, rt, 2–3 h; (2) glutaric anhydride, Et_3N, DMAP, DCM, rt, 6–24 h; (3) phthalic anhydride, Et_3N, DMAP, DCM, rt, 12–15 h.