Bioorganic & Medicinal Chemistry Letters 22 (2012) 801–805

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and evaluation of nitric oxide-releasing DDB derivatives as potential Pgp-mediated MDR reversal agents in MCF-7/Adr cells

Xiaobo Tang [a,b,†], Xiaoke Gu [a,b,†], Hua Ai [c], Guangji Wang [c], Hui Peng [d], Yisheng Lai [a,b], Yihua Zhang [a,b,*]

[a] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China
[b] Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, PR China
[c] Key Lab of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing 210009, PR China
[d] Department of Molecular Immunology, Institute of Basic Medical Sciences, Beijing 100850, PR China

ARTICLE INFO

Article history:
Received 13 September 2011
Revised 29 November 2011
Accepted 13 December 2011
Available online 17 December 2011

Keywords:
Multidrug resistance (MDR)
Pgp inhibitor
DDB
NO-releasing derivatives
MDR reversal agents

ABSTRACT

Novel furoxan-based nitric oxide (NO)-releasing DDB derivatives (**7a–j**) were synthesized. Compounds **7i** and **7j** significantly reversed the resistance of MCF-7/Adr cells to doxorubicin in the combination treatment, and markedly increased the intracellular accumulation of doxorubicin probably via inhibiting Pgp-mediated intracellular drug efflux as well as down-regulating doxorubicin-induced Pgp expression. It was demonstrated that NO released by **7i** and **7j** played an important role in increasing intracellular doxorubicin accumulation and chemo-sensitizing MCF-7/Adr cells to doxorubicin, and the synergic effects of DDB and NO-donor moieties in **7i** and **7j** may contribute to reversing Pgp-mediated MDR in MCF-7/Adr cells to doxorubicin.

Chemotherapy is one of the most important strategies for the treatment of cancers. However, extensive multidrug resistance (MDR) in cancer cells has been a major obstacle to successful cancer chemotherapy.[1] The mechanism underlying which cancer cells develop resistance to anticancer drugs is complicated. To date, one of the best characterized mechanisms of MDR involves the overexpression of ATP-binding cassette (ABC) transporter Pgp (also termed ABCB1, MDR1) on the plasma membrane of tumor cells. Pgp can actively efflux a broad range of structurally unrelated anticancer drugs out of the cell, thereby decreasing their intracellular levels and therapeutic efficacy.[2] As for this aspect, considerable attempts have been made to suppress MDR in tumors by developing Pgp inhibitors during past decades. However, until now, no drug of this class has been approved for many reasons, such as low selectivity, poor potency, inherent toxicity and/or adverse pharmacokinetic interaction with anticancer drugs.[3] Despite these difficulties, there is a clear and urgent requirement for developing effective Pgp inhibitors, and our present work is focused on this purpose.

Dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphen-yl-2,2′-dicarboxylate (DDB) is a synthetic hepatoprotective drug which has been widely used for the treatment of chronic viral hepatitis B with few side effects for more than 20 years in China.[4] Previous reports showed that DDB had anticancer activity not by cytotoxicity but preventing and reversing the development of cancers and inducing differentiation on tumor cells to normal cells.[5,6] Interestingly, recent research demonstrated that DDB was able to reverse Pgp-mediated MDR in vitro and in vivo by increasing intracellular accumulation of anticancer drugs and promoting apoptosis through inhibiting Pgp, and more importantly, there was no pharmacokinetic interaction problem between DDB and anticancer drugs, at least doxorubicin.[4]

Nitric oxide (NO) is a special gaseous molecular functioned as a potent biological mediator in a myriad of physiological and pathological events.[7] Numerous studies have showed that high levels of NO can inhibit the growth of tumor cells and promote their apoptosis by many mechanisms.[8] Indeed, our group's previous reports have revealed that the synthesized NO-releasing compounds have strong anti-proliferative activity against human carcinoma cells in vitro and inhibit cancer cells growth in vivo.[9–12] It has also reported that some NO-donors or NO mimetic agents are able to increase sensitivity of resistant tumor cells to anticancer drugs and attenuate hypoxia-induced drug resistance in solid tumors.[13,14]

Abbreviations: DDB, dimethyl-4,4′-dimethoxy-5,6,5′,6′-dimethylenedioxybiphenyl-2,2′-dicarboxylate; NO, nitric oxide; MDR, multidrug resistance; VER, Verapamil; Dox, doxorubicin; EDCI, 1-(3-dimethyl aminopropyl)-3-ethylcarbodiimide hydrochloride; DMAP, 4-dimethylaminopyridine; MTT, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide; RF, reversal fold; TEER, transepithelial electrical resistance; P_{app}, apparent permeability coefficient; HRP, horseradish peroxidase.

* Corresponding author. Tel./fax: +86 25 83271015.
E-mail address: zyhtgd@163.com (Y. Zhang).

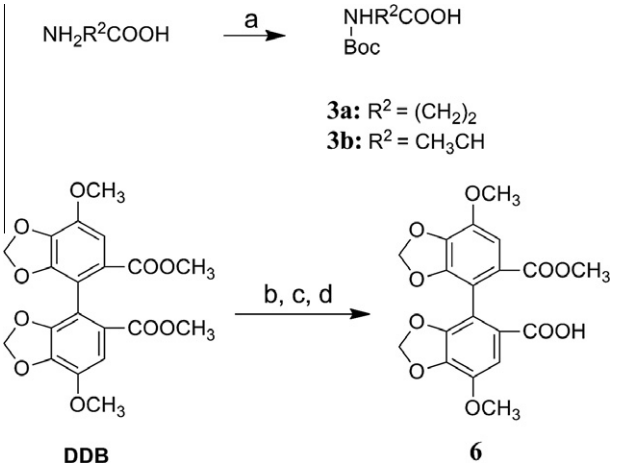
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.12.065

The potential mechanisms include vascular changes that promote blood delivery and tumor oxygenation, and inhibition of key transcription factors such as HIF-1 and NF-κB as well as drug efflux transporters.[15] Furoxans is well known as an important class of NO donors, which can produce high levels of NO in vitro, and inhibit the growth of tumors in vivo.[9,12] In addition, the latest findings documented that NO released by 3-phenylsulfonyl substituted furoxans is able to restore in high degree the anti-proliferative activity of doxorubicin by nitrating tyrosine residues of Pgp in MCDK-MDR1 cancer cells.[16]

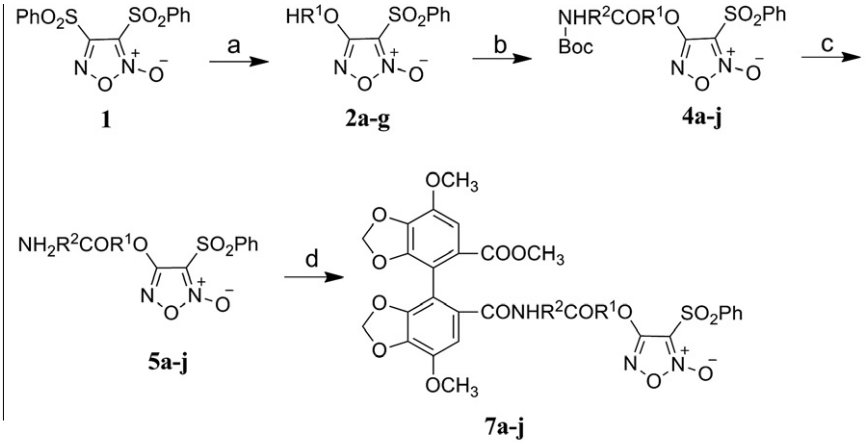
Based on the above investigations, we hypothesized that new types of furoxan/DDB hybrids could produce high levels of NO leading to potent inhibitory activity against human cancer cells and reverse Pgp-mediated MDR in tumor cells. To verify our assumption, ten target compounds (**7a–j**) were designed and synthesized. The anti-proliferative activity of these compounds was assayed, and the compounds **7i** and **7j** containing L-α-alanine as a linker were further investigated as Pgp-mediated MDR reversal agents in MCF-7/Adr cells. Herein, we report the synthesis and biological evaluation of these target compounds.

The synthetic route of **7a–j** is outlined in Scheme 1. Furoxan **1** was synthesized according to the literature.[10] The treatment of **1** with diol or alkanolamine gave corresponding monophenyl-sulfonyfuroxans **2a–g** in 81–90% yields. These substituted-furoxans were reacted with N-protected β-alanine **3a** or L-α-alanine **3b** (Scheme 2) in the presence of EDCI and DMAP in CH_2Cl_2 afforded esters **4a–j** in 68–90% yields. The N-protected compounds **4a–j** were treated with CF_3COOH to provide the intermediates **5a–j**, which were directly condensed with **6**, a monocarboxyl ester of DDB, in the presence of EDCI and DMAP in CH_2Cl_2 to generate the target compounds **7a–j** in 60–82% yields.[17,18] The final products were purified by column chromatography and their structures were characterized by IR, ^{1}H-NMR, MS and elemental analyses.[19]



Scheme 2. Synthesis of compounds **3a**, **3b** and **6**. (a) $(Boc)_2O$, t-BuOH, 2.5% NaOH, rt, 5–10 h; (b) 10% NaOH, reflux, 5 h; (c) $(Ac)_2O$, reflux, 8 h; (d) CH_3OH, reflux, 3 h.

The anti-proliferative activity of individual compounds against human HepG2 cells in vitro was evaluated by MTT assay using 5-fluorouracil (5-FU) as positive control. The IC_{50} values of these compounds are presented in Table 1. It was observed that most of compounds displayed strong inhibitory activity against human HepG2 cells, superior to DDB and even stronger than 5-FU. Interestingly, compounds **7i** and **7j** containing L-α-alanine as a linker showed little inhibitory effect on MCF-7 cells and its



2a: $R^1 = O(CH_2)_2$ **2b:** $R^1 = O(CH_2)_3$ **2c:** $R^1 = O(CH_2)_4$ **2d:** $R^1 = NH(CH_2)_2$ **2e:** $R^1 = O(CH_2)_2O(CH_2)_2$ **2f:** $R^1 = OCH(CH_3)(CH_2)_2$ **2g:** $R^1 = OC(CH_3)_2(CH_2)_2$

4-7a:	$R^1 = O(CH_2)_2$	$R^2 = (CH_2)_2$	**4-7f:**	$R^1 = OCH(CH_3)(CH_2)_2$	$R^2 = (CH_2)_2$
4-7b:	$R^1 = O(CH_2)_3$	$R^2 = (CH_2)_2$	**4-7g:**	$R^1 = OC(CH_3)_2(CH_2)_2$	$R^2 = (CH_2)_2$
4-7c:	$R^1 = O(CH_2)_4$	$R^2 = (CH_2)_2$	**4-7h:**	$R^1 = O(CH_2)_3$	$R^2 = CH_3CH$
4-7d:	$R^1 = NH(CH_2)_2$	$R^2 = (CH_2)_2$	**4-7i :**	$R^1 = O(CH_2)_4$	$R^2 = CH_3CH$
4-7e:	$R^1 = O(CH_2)_2O(CH_2)_2$	$R^2 = (CH_2)_2$	**4-7j :**	$R^1 = O(CH_2)_2O(CH_2)_2$	$R^2 = CH_3CH$

Scheme 1. Synthesis of compounds **7a–j**. Reagents and conditions: (a) diol or alkanolamine, THF, 25% NaOH, rt, 0.5 h; (b) **3a** or **3b**, EDCI, DMAP, CH_2Cl_2, rt, 4 h; (c) CF_3COOH, rt, 2 h; (d) **6**, EDCI, DMAP, CH_2Cl_2, rt, 5 h.