Bioorganic & Medicinal Chemistry 24 (2016) 2971–2978

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Antiproliferative activity and apoptosis inducing effects of nitric oxide donating derivatives of evodiamine

Nan Zhao [a,†], Kang-tao Tian [a,†], Ke-guang Cheng [b], Tong Han [a], Xu Hu [a], Da-hong Li [a,b,*], Zhan-lin Li [a], Hui-ming 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, China
[b] State Key Laboratory for the Chemistry and Molecular Engineering of Medicinal Resources, and School of Chemistry and Pharmacy, Guangxi Normal University, 15 Yucai Road, Guilin 541004, China

ARTICLE INFO

Article history:
Received 27 February 2016
Revised 30 April 2016
Accepted 2 May 2016
Available online 3 May 2016

Keywords:
Nitric oxide
Evodiamine
Antiproliferative activity
Apoptosis

ABSTRACT

The first series of nitric oxide donating derivatives of evodiamine were designed and prepared. NO releasing ability of all target derivatives was evaluated in BGC-823, Bel-7402 and L-02 cells. The cytotoxicity was evaluated against three human tumor cell lines (Bel-7402, A549 and BGC-823) and normal human liver cells L-02. The nitrate derivatives **11a** and **11b** only exhibited moderate activity and furoxan-based derivatives **13a–c**, **14a** and **14b** showed promising activity. **13c** showed good cytotoxic selectivity between tumor and normal liver cells and was further investigated for its apoptotic properties on human hepatocarcinoma Bel-7402 cells. The molecular mode of action revealed that **13c** caused cell-cycle arrest at S phase and induced apoptosis in Bel-7402 cells through mitochondria-related caspase-dependent pathways.

1. Introduction

Natural products or their derivatives take an active role in the development of new therapeutic drugs.[1] Evodiamine (**1**, Scheme 1), a quinazolinocarboline alkaloid isolated from the fruits of *Euodia rutaecarpa*, possesses many biological effects, such as antitumor,[2–5] anti-inflammation,[6–8] antiobesity,[9–11] and so on.[12–14] Particularly, numerous studies have comprehensively demonstrated that **1** exhibited considerable cytotoxicity on a wide variety of human cancer cell lines,[15–17] and apoptosis inducing ability to suppress the proliferation of tumor cells by various mechanisms,[18–20] such as, PI3K/Akt/caspase, Fas-L/NF-κB signaling pathways,[21] caspase-dependent and -independent pathways,[22,23] and MTDH-dependent signaling pathway.[24] Though there were a great deal of reports which clarified the antiproliferation and apoptosis functions of evodiamine, it was unpractical to develop it directly as clinic agents owing to its moderate anticancer activity.[25] Besides, hepatotoxicity caused by the plant *E. rutaecarpa* had not received serious attention providing new challenges.[26] Some promising derivatives of **1** had already been reported,[27–30] including 3,4,10,13-position modified evodiamine,[27] carboxyl derivatives at position 7 targeting topoisomerase I and sirtuins,[28] a diverse library containing 11 evodiamine-inspired novel scaffolds and their derivatives as multitargeting antitumor agents,[29] hybrid molecules of 3-amino-10-hydroxylevodiamine and SAHA as triple inhibitors of topoisomerase I/II and HDAC,[30] and so on. Because of its broad-spectrum and multitargeting antitumor profile, evodiamine represented a good lead; more work of structure modification was still in urgent need to be taken out.

Nitric oxide (NO) is a small and reactive molecule, which has various physiological and biological properties.[31] High concentration of NO has shown great potential in inhibiting carcinogenesis and tumor growth by inducing tumor cell apoptosis, inhibiting tumor metastasis, and so on.[32,33] Unfortunately, the delivery of gaseous NO to tumor directly is not really effective due to its short half-life and chemical instability.[34,35] NO donors, capable of producing a sustained release with a wide range of half-time lives, and a predictable estimated dose had become useful tools to study the biological properties of NO in cells and in vivo models of carcinogenesis. Recently, great deals of NO donor hybrids spring up, which primarily served as anticancer drugs.[36–38] We were very interested in what aspects of druggability these hybrids would performance.

Inspired by the above reasons, a novel series of evodiamine derivatives bearing NO-donating groups (organic nitrate or furoxan)

* Corresponding author. Tel.: +86 24 23986465.
E-mail addresses: lidahong0203@163.com (D.-h. Li), huimhua@163.com (H.-m. Hua).
† Co-first authors.

http://dx.doi.org/10.1016/j.bmc.2016.05.001
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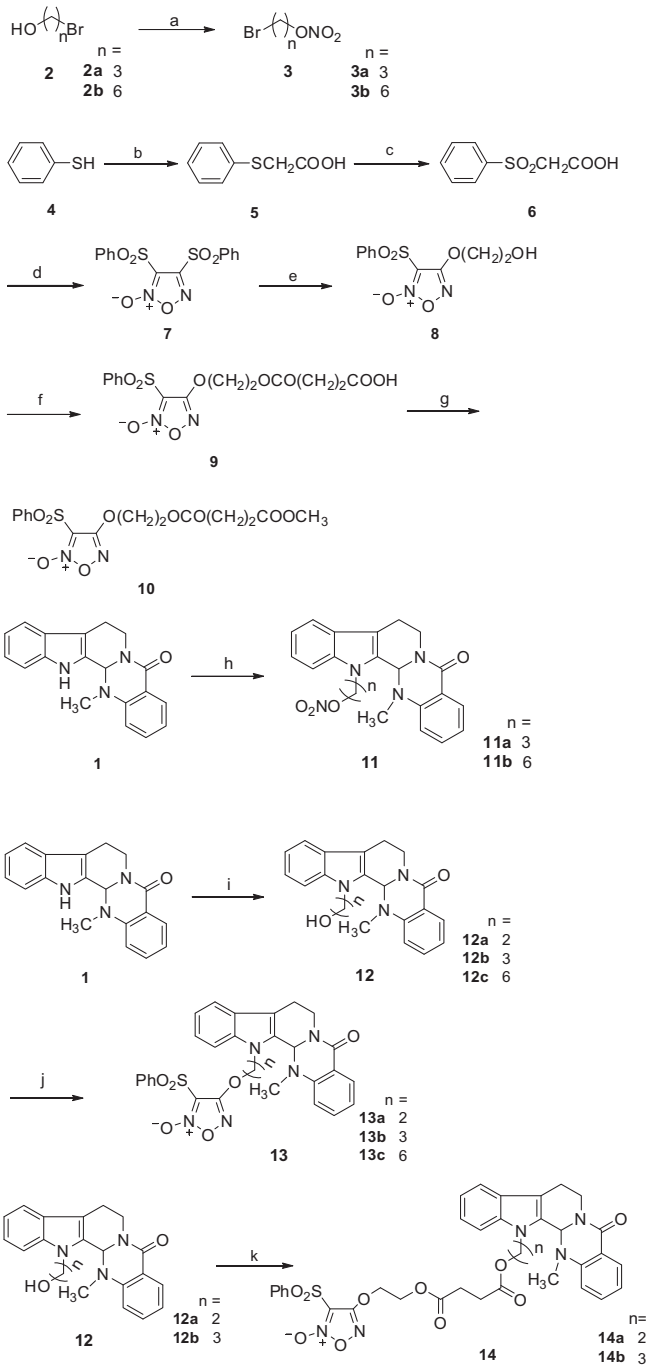

Scheme 1. Synthetic routine of NO-releasing evodiamine derivatives **11**, **13** and **14**. Reagents and conditions: (a) fuming HNO_3, H_2SO_4, 0 °C, 3 h; (b) $ClCH_2COOH$, NaOH (aq), 140 °C, 2 h; (c) 30% H_2O_2, AcOH, rt, 3 h; (d) fuming HNO_3, 90 °C, 4 h; (e) $HOCH_2CH_2OH$, THF, 30% NaOH, 0 °C, 4–8 h; (f) triethylamine, succinic anhydride, DMAP, rt, 1 h; (g) $TMSCHN_2$, MeOH, 0 °C, 10 min; (h) **3**, NaH, DMF, rt, 1.5 h; (i) $HO(CH_2)_nBr$, NaH, DMF, rt, 3 h; (j) **7**, DBU, CH_2Cl_2, −15 °C, 3 h; (k) **9**, EDCI, DMAP, rt, 12 h.

were designed and synthesized at *N*-13. The antiproliferative activity against human hepatoma cells (Bel-7402), human non-small-cell lung cancer cells (A549), human gastric carcinoma cells (BGC-823) and human normal liver cells (L-02) was evaluated. The NO releasing property was also measured. Typical selected compound **13c** was further investigated for its apoptotic properties on human hepatocarcinoma Bel-7402 cells, in order to gain a better understanding of the mode of action. The effects of apoptosis, cell cycle arrest, and mitochondrial membrane potential were also disclosed.

2. Results and discussion

2.1. Chemistry

The procedures for the synthesis of NO-releasing evodiamine derivatives were illustrated in Scheme 1. The corresponding bromohydrin **2** was treated with fuming HNO_3 and concentrated H_2SO_4, which afforded nitrate **3**. The reaction of thiophenol **4** with chloroacetic acid in the presence of sodium hydroxide solution yielded the thiophenylacetic acid **5**, which was further oxidated with 30% H_2O_2 and AcOH, generating the oxidation product **6**. **6** was treated with fuming HNO_3 at 90 °C, which lead to **7**. 3,4-Dibenzenesulfonyl furoxan **7** was treated with ethanediol in the presence of 30% NaOH in THF to offer **8**. Treatment of **8** with triethylamine, succinic anhydride, and 4-dimethylaminopyridine (DMAP) produced the intermediate **9**. **9** was reacted with $TMSCHN_2$ to get **10**. For **11a**–**b**, the reaction of **1** with **3a** or **3b**, in the presence of NaH, was carried out in DMF. **1** was treated with bromohydrin in the presence of NaH and DMF to offer **12a**–**c**, and then **7** was added in the presence of DBU in CH_2Cl_2 to give evodiamine–furoxan hybrids **13a**–**c**. Intermediate **11** was reacted with **9** to afford another series of evodiamine–furoxan hybrids **14** with long linkage.

2.2. NO releasing ability

In order to investigate whether the evodiamine derivatives including NO donor possessed the ability to release NO and if there were any differences of NO releasing in tumor and normal cells, Griess assay was carried out in BGC-823, Bel-7402 and L-02 cell lines. As shown in Table 1, all the derivatives released more than 75 μM/L of NO at the time point of 1 h in BGC-823 and Bel-7402 cells, and less than 28.12 μM/L of NO in L-02 cells. This would be caused of special chemical environment (such as low pH) in tumor cells. In further investigation, control release of NO might be a good topic. Almost all target compounds released a little more NO in BGC-823 cells than Bel-7402 cells (except **11b**). Of all the derivatives, **11b** released the highest amount of NO of 104.18 μM/L in Bel-7402 cells at the time point of 1 h, while in L-02 cells the least NO of 6.59 μM/L was produced by **14a**.

2.3. Antiproliferative activity

The antiproliferative activity of target compounds was preformed on BGC-823, A549, Bel-7402, and L-02 cell lines by the standard MTT method. The results were summarized in Table 2. The nitrate derivatives **11a** and **11b** only exhibited moderate activity against Bel-7402 cells with IC_{50} values of 12.82 and 28.79 μM, respectively. And shorter linkage (**11a**) was favorable. As for furoxan-based derivatives **13a**–**c**, **14a** and **14b**, they showed very promising activity against BGC-823 cells with IC_{50} values ranging from 0.02 to 0.08 μM. These results revealed that the synthetic NO donating derivatives of **1** were very sensitive to BGC-823 cells. **13a** was the most potent one against A549 and Bel-7402 cells with IC_{50} values of 0.23 and 0.55 μM, and other compounds were weaker than parent compound **1**. In L-02 cells, significant differences were observed that **13a** still exhibited the strongest cytotoxicity with IC_{50} value of only 0.02 μM and no obvious antiproliferative activity (>100 μM) was exhibited by **13c**. **14a** with a linker of 2 carbons showed IC_{50} values of 0.02, 3.04 and 1.57 μM against three tumor cell lines, respectively, which was stronger than **14b** of 3 carbons with IC_{50} values of 0.06, 5.32 and 4.65 μM.