Bioorganic & Medicinal Chemistry Letters xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel nitric oxide-releasing spirolactone-type diterpenoid derivatives with in vitro synergistic anticancer activity as apoptosis inducer

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ARTICLE INFO

Article history:
Received 24 April 2016
Revised 20 July 2016
Accepted 25 July 2016
Available online xxxx

Keywords:
NO-releasing
Spirolactone-type
Diterpenoid
Oridonin
Antiproliferative

ABSTRACT

Herein, we reported the cytotoxicity, NO-releasing property, and apoptosis induced ability of two series of novel nitric oxide-releasing spirolactone-type diterpenoid derivatives (**10a–f** and **15a–f**). All the title compounds were more potent than oridonin (**7**) and parent compound (**9** or **14**) against human tumor Bel-7402, K562, MGC-803 and CaEs-17 cells. SARs were concluded based on above data. Compound **15d** exhibited the strongest antiproliferative activity with the IC_{50} of 0.86, 1.74, 1.16 and 3.75 μM, respectively, and could produce high level (above 25 μM) of NO at the time point of 60 min. Further mechanism evaluation showed that **15d** could induce S phase cell cycle arrest and apoptosis at low micromolar concentrations in Bel-7402 cells via mitochondria-related pathways. It was expected that the remarkable biological profile of the synthetic NO-releasing spirolactone-type diterpenoid analogs make them possible as promising candidates for the development of anticancer agents.

Nitric oxide (NO) is an important, active, and small molecule.[1] It plays important roles in various physiological processes, including neuromodulation, vasorelaxation, and biodefence, it may also be involved in the pathophysiology of diseases such as cancer, schizophrenia and Alzheimer's disease. Based on previous research, high concentration of NO and its metabolic derivatives, the reactive nitrogen species (RNS) could modify functional proteins by *S*-nitrosylation, nitration, disulfide formation leading to bioregulation, inactivation, and cytotoxicity particularly to tumor cells by apoptosis and blocking cell migration.[2–6] Therefore, methods for precisely controlled release of NO were required for research purposes and might ultimately be clinically relevant. Since NO was unstable under ambient conditions, compounds (NO donors) that release NO in situ have been developed and employed for the research. NO donors could release a certain amount of NO by the action of enzymes and/or non-enzymes. Several kinds of NO donors were developed, such as organic nitrates, metal-NO complexes, nitrosothiols, and so on.[7,8] With the development of NO donors, NO-donating drugs emerged and developed rapidly. NO donor hybrid compounds had come into focus in the treatment of tumor because NO was a key signaling molecule involved in the death and apoptosis of tumor cells.[7–12]

Diterpenoids were well-known to produce bioactive molecules.[13–15] Among which, spirolactone-type 6,7-*seco*-kaurane diterpenoids had unique chemical skeletons and exhibited significant

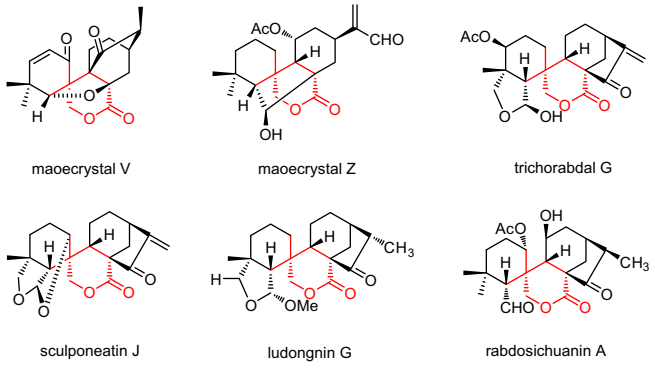

Figure 1. The structures of natural spirolactone-type 6,7-*seco*-kaurane diterpenoids.

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http://dx.doi.org/10.1016/j.bmcl.2016.07.059
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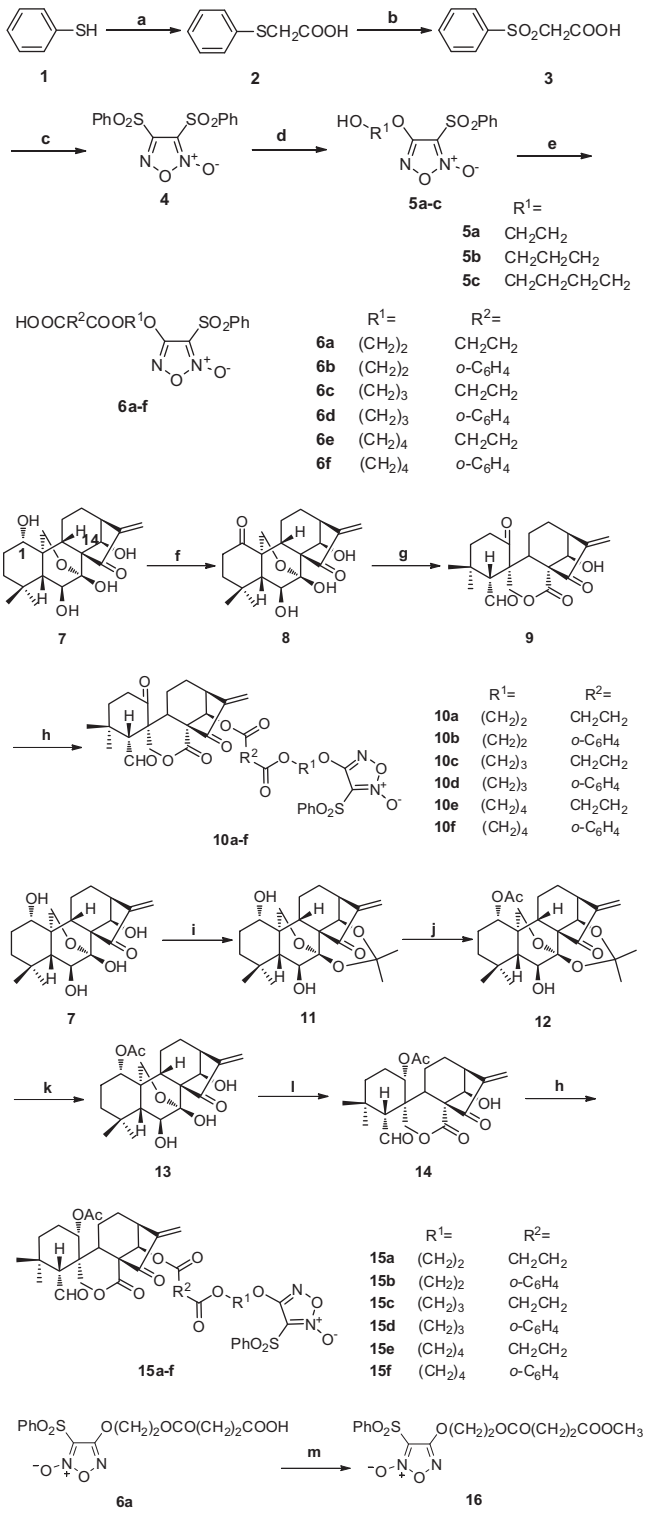

Scheme 1. The synthetic routine of target NO releasing spirolactone-type 6,7-*seco*-kaurane diterpenoid derivatives. Reagents and conditions: (a) chloroactic acid, NaOH (aq), 60 °C; (b) 30% H_2O_2, glacial acetic acid, rt; (c) fuming HNO_3, glacial acetic acid, 60 °C; (d) corresponding diol, NaOH (aq), THF, rt; (e) anhydride, DMAP/TEA, DCM, rt; (f) Jones reagent, acetone, isopropanol, 0 °C; (g) $Pb(OAc)_4$, Na_2CO_3, THF, rt; (h) **6a–f**, EDCI, DMAP, DCM, rt; (i) 2,2-dimethoxypropane, acetone, TsOH, 56 °C; (j) Ac_2O, TEA, DMAP, rt; (k) 10% HCl, THF, rt; (l) $Pb(OAc)_4$, Na_2CO_3, THF, rt; (m) $TMSCHN_2$, MeOH, 0 °C, 10 min.

activities, such as maoecrystal V,[16] maoecrystal Z,[17] trichorabdal G,[18] sculponeatin J,[19] ludongnin G,[20] rabdosichuanin A,[21] and so on (Fig. 1). These hot off the press spirolactone-type diterpenoids with a lot of stereogenic centers and complex ring systems had attracted much attention from chemists in the field of total synthesis.[22–25] They also aroused our curiosity for further medicinal chemistry investigation.

Table 1
Antiproliferative activity of target compounds **10a–f** and **15a–f** against four human cancer cell lines (IC_{50}[a] μM)

Compd	K562	MGC-803	CaEs-17	Bel-7402
7	4.59 ± 0.22	5.47 ± 0.48	12.16 ± 0.67	7.56 ± 0.32
9	2.92 ± 0.23	4.27 ± 0.32	5.85 ± 0.27	5.03 ± 0.37
10a	2.40 ± 0.07	2.43 ± 0.12	5.21 ± 0.24	1.50 ± 0.13
10b	2.04 ± 0.17	1.93 ± 0.10	4.67 ± 0.10	0.96 ± 0.02
10c	2.34 ± 0.23	2.89 ± 0.13	5.39 ± 0.25	1.49 ± 0.05
10d	1.97 ± 0.13	1.63 ± 0.12	4.12 ± 0.27	0.93 ± 0.04
10e	1.99 ± 0.11	1.80 ± 0.17	4.83 ± 0.33	1.24 ± 0.11
10f	1.90 ± 0.06	1.87 ± 0.18	4.66 ± 0.16	1.03 ± 0.08
14	9.11 ± 0.36	28.54 ± 2.58	66.94 ± 4.05	69.40 ± 3.72
15a	2.48 ± 0.20	2.73 ± 0.20	5.88 ± 0.32	1.53 ± 0.14
15b	2.03 ± 0.12	1.68 ± 0.09	4.51 ± 0.19	0.92 ± 0.06
15c	2.91 ± 0.29	3.09 ± 0.15	6.26 ± 0.39	1.83 ± 0.12
15d	1.74 ± 0.07	1.16 ± 0.04	3.75 ± 0.08	0.86 ± 0.01
15e	2.88 ± 0.18	3.03 ± 0.22	6.20 ± 0.27	1.74 ± 0.16
15f	1.79 ± 0.13	1.56 ± 0.14	4.40 ± 0.22	0.91 ± 0.07
16	19.65 ± 0.87	24.57 ± 1.34	22.60 ± 1.41	27.86 ± 0.76
Taxol	0.38 ± 0.02	0.83 ± 0.07	0.51 ± 0.02	1.95 ± 0.17

[a] IC_{50}: concentration that inhibits 50% of cell growth. Results are expressed as the mean ± S.D. of three independent experiments.

On the basis of the above mentioned reasons, as a part of further development of our research work on the design of new NO donor/diterpenoid derivatives, two series of spirolactone-type 6,7-*seco*-kaurane diterpenoid and NO donor motif hybrids were synthesized. Relevant commercial available resources were used for acquiring complicated target natural product skeletons by passing traditional isolation and the de novo synthetic method. Then the antiproliferative activity of target compounds was tested by MTT assay. Furthermore, the influence of cell cycle progression and effects on apoptosis and mitochondrial membrane potential by representative compound **15d** in Bel-7402 cells were disclosed.

The synthesis of substituted furoxans (**6a–i**) was illustrated in Scheme 1. Starting from benzenethiol (**1**), diphenylsulfonylfuroxan (**4**) was got and then treated with corresponding diol (ethane-1,2-diol, propane-1,3-diol, and butane-1,4-diol) to afford monophenylsulfonylfuroxans (**5a–c**). Key intermediate furoxan-based NO donors **6a–f** were obtained from the condensation of **5a–c** and anhydride (succinic anhydride or phthalic anhydride).[10] Selected oxidation of **7** with Jones Reagent gave 1-oxo derivative **8**, and then lead tetraacetate was added in the presence of sodium carbonate in THF to get spirolactone-type diterpenoid **9** in high yields. Treatment of **7** with 2,2-dimethoxypropane (DMP) in the presence of TsOH in anhydrous acetone afforded **11**, which, upon reaction with Ac_2O/Et_3N in CH_2Cl_2, yielded 1-*O*-acetyl derivative **12**. Deprotection with 10% HCl gave compound **13** in quantitative yields, which could be converted to spirolactone-type diterpenoid **14** by oxidation using lead tetraacetate in the presence of sodium carbonate in THF in high yields.[26,27] **9** and **14** with a hydroxyl group at 14-position were good building blocks to synthesize spirolactone-type diterpenoid analogs. Target novel NO donor/spirolactone-type 6,7-*seco*-*ent*-kaurane diterpenoid hybrids **10a–f** and **15a–f** were designed and synthesized from **9** and **14** accordingly with NO donors **6a–f** in the presence of DMAP/EDCI in DCM at room temperature for 8–12 h. Flash chromatography could only be taken at the last step of the synthetic route of each target compound.[28] **6a** was reacted with $TMSCHN_2$ to get **16**.

The antiproliferative activity of **10a–f** and **15a–f** against four different kinds of human cancer cell lines (K562 leukemia cell line, MGC-803 gastric cancer cell line, CaEs-17 esophageal cancer cell