Bioorganic & Medicinal Chemistry 23 (2015) 5056–5060

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis and bioactivity of a Goralatide analog with antileukemic activity

Zhiliang Li [a], Iryna O. Lebedyeva [a,b], Vita M. Golubovskaya [c], William G. Cance [c], Khalid A. Alamry [d], Hassan M. Faidallah [d], C. Dennis Hall [a,*], Alan R. Katritzky [a,†]

[a] Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, United States
[b] Department of Chemistry and Physics, Georgia Regents University, 1120 15th Street SCI W3005, Augusta, GA 30912, United States
[c] Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY 14263, United States
[d] Chemistry Department, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Article history:
Received 2 February 2015
Revised 13 April 2015
Accepted 21 April 2015
Available online 14 May 2015

Keywords:
Anticancer
Antileukemic
Goralatide
Peptide
Peptidomimetic

ABSTRACT

Natural tetrapeptide Goralatide (AcSDKP) is a selective inhibitor of primitive haematopoietic cell proliferation. It is not stable in vivo and decomposes within 4.5 min when applied to live cells. In this work we developed an analog of Goralatide that exhibits cytotoxicity towards human myeloid HL-60, HEL, Nalm-6 leukemia cells, endothelial HUVEC, glioblastoma U251 and transformed kidney 293T cells. The Goralatide analog showed significant stability in organic solution with no tendency to degrade oxidatively.

1. Introduction

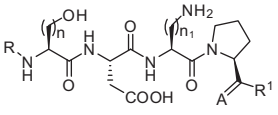
Goralatide (AcSDKP) (**1a**, Fig. 1) first isolated from fetal calf bone marrow,[1] shows selective protection of human hematopoietic progenitor cells during chemotherapy.[2] In vivo, Goralatide prevents murine hematopoietic stem cells and progenitors from entering into *S*-phase following administration of lethal doses of anti-cancer drugs[3] or irradiation.[4,5] High levels of AcSDKP **1a** are markers of thymosin β4 gene overexpression found in various leukemic cell samples.[6] Goralatide is a natural and specific substrate of the N-terminus active site of human angiotensin-converting enzyme.[7–10] New properties have recently been added to the Goralatide bioactivity profile that enhance the myelopoietic response to granulocyte-macrophage colony-stimulating factor (GM-CSF),[11] protect stem cells from hyperthermic damage,[12] and block doxorubicin-induced toxicity in vivo.[2] Goralatide also prevents skin and hair aging,[13] stimulates angiogenesis[14] and shows anti-inflammatory activity.[15,16]

Despite a wide range of possible bioapplications, Goralatide **1a** (Fig. 1) has an extremely short half-life of 4.5 min in plasma.[17,18] Consequently AcSDKP-derived analogs have been designed that are stable in the blood[18–22] and inhibit primitive hematopoietic cell proliferation.[17] Gaudron et al. synthesized a series of AcSDKP peptidomimetic analogs such as AcSerΨ(CH_2-NH)Asp-LysPro, Ac-Ser-AspΨ(CH_2NH)-LysPro, Ac-Ser-Asp-LysΨ(CH_2-N)Pro, the C-terminus modified tetrapeptide Ac-Ser-Asp-Lys-Pro-NH_2[17,18] (**1b**, Fig. 1) and the chirally distinct mimetic AcSDDKP.[17] These peptidomimetics reduce in vitro the proportion of murine colony-forming units granulocyte/macrophage in *S*-phase and inhibit entry into the cycle of high proliferative colony-forming cells.[17] They also show resistance to an angiotensin-converting enzyme which prevents rapid elimination of peptidomimetics from plasma.[19] Thierry et al. synthesized a number of AcSDKP analogs (NAcSD, NAcSEKP, NAcADKP, NAcSDβKP) and molecular fragments (SDK, SD, DKP, DK, SDKP) to identify the minimal sequence that is responsible for the biological activity of Goralatide. Synthetic analogs and di-, or tri-peptide sequences were then studied for cellular interactions between T-cell and erythrocytes in Rosette Formation.[20] The same group reported syntheses of several R substituted AcSDKP analogs, where R = Boc, Fmoc, Ant, Suc, coumarin (**1d**–**h**, Fig. 1).

All of these analogs retained the antiproliferative activity and toxicity of Goralatide. The change of L-Ser for *homo*-L-Ser (**1c**) and L-Lys for L-Arg in the original molecule shows that polar groups in the molecule are critical for the expression of biological

* Corresponding author. Tel.: +1 (352) 392 0554; fax: +1 (352) 392 9199.
E-mail address: charlesdennishall@gmail.com (C. Dennis Hall).
† Deceased author.

http://dx.doi.org/10.1016/j.bmc.2015.04.061
0968-0896/



1a: R = Acetyl, R^1 = OH, n = 1, n_1 = 4
b: R = Acetyl , R^1 = NH_2, n = 1, n_1 = 4
c: R = Acetyl, R^1 = OH, n = 2, n_1 = 4
d: R = Boc, R^1 = OH, n = 1, n_1 = 4
e: R = Fmoc, R^1 = OH, n = 1, n_1 = 4
f: R = Ant, R^1 = OH, n = 1, n_1 = 4
g: R = Suc, R^1 = OH, n = 1, n_1 = 4
h: R = Coumarin, R^1 = OH, n = 1, n_1 = 4

Figure 1. Reported synthetic structural analogs of Goralatide.

activity.[21] Furthermore, coumarin-SDKP (**1h**, Fig. 1), allows photochemical determination of angiotensin enzyme activity in plasma.[22]

2. Results and discussion

In this work we synthesized an analog of natural tetrapeptide Goralatide[23] (**1a**) which showed significant antileukemic activity and stability in a number of organic solvents (DMSO, MeOH, $CDCl_3$). The activity of Goralatide analog **11** was studied on human myeloid HL-60, HEL and Nalm-6 leukemia cells, U251 glioblastoma astrocytoma, 293T kidney and HUVEC endothelial cells.

2.1. Chemistry

N- and *O*-protected tetrapeptide **11** was synthesized in six steps. First, the amino group of *O*-(*tert*-butyl)-L-serine **2** was acylated to give **3** in 88% yield. The carboxy group of *N*-acetyl-*O*-(*tert*-butyl)-L-serine **3** was then activated with benzotriazole to afford benzotriazolide **4** (85%). Subsequent reaction with mono-*O*-protected aspartic acid **5** gave dipeptide **6** (64%, Scheme 1). Intermediate dipeptide **6** was then used as follows to construct the core of protected Goralatide tetrapeptide precursor **11** (Scheme 2).

Coupling of *N*,*N*′-diprotected L-lysine **7** with benzyl L-prolinate **8** gave dipeptide benzyl ester **9** in 75% yield. After the Boc group of **9** was removed, trifluoroacetate dipeptide **10** was obtained and was subsequently reacted with dipeptide **6** (HOBt/EDCI, rt 12 h, 80%) to give *O*- and *N*-protected NAcSDKP precursor **11** which, after two deprotection steps, gave final product **1a**.

First, the benzyl ester of L-proline and Cbz protection of L-lysine were removed by Pd/C 10% wt under H_2. Intermediate **12** showed low stability in organic solvents and therefore acidolysis of **12** was performed rapidly after filtering and drying the reaction mixture to afford **1a**[23] (Scheme 2).

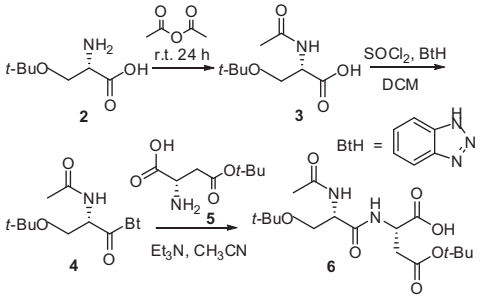
2.2. Evaluation of cytotoxicity in cell lines

In order to explore the bioactivity potential of Goralatide analog **11**, we tested it on the viability of human leukemia cells HL-60, HEL, Nalm-6 and HUVEC endothelial cells. The effect of the *O*, *N*-protected tetrapeptide **11** on survival of glioblastoma U251 and kidney 293T cells by clonogenicity assay was also studied and it was found that **11** decreased the clonogenicity of U251 and 293T cancer cells (Fig. S1, SI).

To test the effect of **11** on cell viability, we performed MTT assay on HL-60 cancer cells (Fig. 2). Tetrapeptide **11** showed a significant decrease in the survival of leukemia cells, compared to the DMSO control.

An analogous toxicity effect for **11** was observed by a viability assay on Nalm-6 cells (Fig. 3) and HEL cells (Fig. 4).

Tetrapeptide **11** decreases HL-60 viability in a dose-dependent manner. The HL-60 cells were treated with different doses of **11** for 24 h and a MTT assay was performed as described in Section 4. The inhibitory activity of **11** on HL-60 cells was also observed by MTT assay in Nalm-6 and HEL cell lines.



Scheme 1. Synthesis of an *O*- and *N*-protected L-Ser-L-AspOH **6**.

Next, we tested the effect of **11** on the viability of endothelial HUVEC cells. It significantly decreased viability of HUVEC cells in a dose-dependent manner (Fig. 5). Tetrapeptide **11** was incubated at different doses with HUVEC cells for 24 h to perform MTT assay $^{*}p<0.05$, Students' *t*-test.

We ran analogous studies on Goralatide **1a** and it had no inhibitive effect on the cancer cell lines employed in the study (Fig. S2, SI). These results are in good agreement with previous reports[9,10] on selectivity towards inhibition of hematopoietic stem cells and effects on cancer cells.

Goralatide **1a** showed decomposition within 12 h in organic solvents (DMSO, $CDCl_3$, MeOH) which complies with the data on the low stability of NAcSDKP in vivo caused by a rapid Asp-Lys cleavage.[2] Tetrapeptide **11** showed no signs of decomposition after being treated with such solvents as DMSO, MeOH, $CDCl_3$ for 7 days.

3. Conclusion

In summary, a stable Goralatide (AcSDKP) analog has been synthesized and its anticancer activity studied. In vitro cytotoxicity assays demonstrated that *N*-, *O*-deprotected AcSDKP analog **11** effectively kills a range of cancer cells. It showed highest cytotoxicity towards glioblastoma U251 and kidney 293T cells. It was also effective against HL-60, Nalm-6 and HEL leukemia cells and HUVEC endothelial cells at a concentration of 50 μM and towards Nalm-6 leukemia cells at a concentration of 20 μM. The high anticancer activity profile of **11** suggests that it could be developed into a novel anti-cancer and anti-leukemic drug. The molecular mechanisms responsible for the antileukemic activity of **11** are currently under investigation and further novel Goralatide analogs with antileukemic mode of action are also being developed.

4. Experimental section

4.1. Materials and methods

All reagents were purchased from commercial sources and used as received unless otherwise indicated. The products were purified by column chromatography on silica gel (300–400 mesh). Melting points were determined on a capillary point apparatus equipped with a digital thermometer. NMR spectra were recorded in $CDCl_3$, DMSO-d_6 or CD_3OD on Mercury or Gemini NMR spectrometers operating at 300 MHz for ^1H (with TMS as an internal standard) and 75 MHz for ^{13}C. Elemental analyses were performed on a Carlo Erba-EA1108 instrument. High Resolution Mass Spectra were recorded using Thermo Scientific LCQ Ion Trap. For biological evaluation Goralatide analog **11** was dissolved in DMSO at 25 mM and kept at −20 °C.