Bioorganic & Medicinal Chemistry Letters 26 (2016) 3122–3126

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synergistic potentiation of (−)-lomaiviticin A cytotoxicity by the ATR inhibitor VE-821

Laureen C. Colis [a], Seth B. Herzon [a,b,*]

[a] Department of Chemistry, Yale University, New Haven, CT 06520, United States
[b] Department of Pharmacology, Yale School of Medicine, New Haven, CT 06520, United States

ARTICLE INFO

Article history:
Received 10 April 2016
Revised 28 April 2016
Accepted 29 April 2016
Available online 30 April 2016

Keywords:
Chemotherapy
DNA
Natural product
Cancer
Lomaiviticin
Synergism

ABSTRACT

(−)-Lomaiviticin A (**1**) is a cytotoxic bacterial metabolite that induces double-strand breaks in DNA. Here we show that the cytotoxicity of (−)-lomaiviticin A (**1**) is synergistically potentiated in the presence of VE-821 (**7**), an inhibitor of ataxia telangiectasia and Rad3-related protein (ATR). While 0.5 nM **1** or 10 μM **7** alone are non-lethal to K562 cells, co-incubation of the two leads to high levels of cell kill (81% and 94% after 24 and 48 h, respectively). Mechanistic data indicate that cells treated with **1** and **7** suffer extensive DNA double-strand breaks and apoptosis. These data suggest combinations of **1** and **7** may be a valuable chemotherapeutic strategy.

(−)-Lomaiviticin A (**1**) is an antiproliferative bacterial metabolite with half maximal inhibitory potencies in the low nanomolar–picomolar range against a panel of cultured human cancer cell lines (Fig. 1).[1] The exquisite cytotoxicity of **1** derives from induction of DNA SSBs and DSBs in tissue culture.[2] The kinetics of DNA DSB induction and PI3KKs pathways-dependent phosphorylation of histone H2AX by **1** have been analyzed.[2b] Reduction of histone H2AX phosphorylation induced by **1** was observed in K562 cells pretreated with inhibitors (KU55933,[3] caffeine,[4] and NU-7441[5]) of the major PI3KKs ATM, ATR, and DNA-PK, respectively. In addition, **1** displayed selective toxicity toward BRCA2-, PTEN-, KU80-, DNA-PKcs, and ATM-deficient cell lines, all of which have been implicated in DSB repair.[2a,b] BRCA2-deficient VC8 and PTEN-deficient U251 cell lines are particularly sensitive to **1** (LC_{50} = 1.5 ± 0.5 and 2.0 ± 0.6 pM, respectively) with selectivities of >11.6 versus the isogenic cell lines transfected with and expressing functional BRCA2 and PTEN genes.[2b] Collectively, these results indicate activation of NHEJ[6] and HR[7] repair, the two canonical pathways by which DNA DSBs are ameliorated, in cells treated with **1** and suggest acceptable therapeutic indices may be attainable when **1** is applied toward NHEJ- or HR-deficient tumors.

These results led us to question if sensitization could be induced pharmacologically using small molecule inhibitors of DNA repair. The application of DNA repair inhibitors as chemo- and radiosensitizers is an active area of research, and several combinations of repair inhibitors and DNA damaging agents (small molecules, IR) are undergoing clinical evaluation.[8] Synergistic potentiation of **1** by DNA repair inhibitors could decrease required doses, resulting in fewer off-target effects and higher therapeutic indices across a range of tumor types, including DSB repair-proficient tumors. Herein, we report an evaluation of combinations of **1** and six inhibitors of DNA DSB repair. We report the discovery that the ATR inhibitor VE-821 (**7**) synergistically potentiates the cytotoxic activity of **1** in K562 cells. Combinations of **7** and **1** induce high levels of cell kill concentrations at which either agent alone is non-lethal.

We investigated six small molecules (Fig. 1) that disrupt essential factors in DNA repair. AZD-2281 (Olaparib, **2**) is an inhibitor of PARP-1 (IC_{50} = 5 nM) and PARP-2 (IC_{50} = 1 nM).[9] Following DNA damage, PARP-1 binds to DNA breaks leading to initiation of BER.[10] BEZ-235 (Dactolisib, **3**) was originally designed as a

Abbreviations: ATR, ataxia telangiectasia and Rad3-related protein; SSB, single-strand break; DSB, double-strand break; PI3KK, phosphatidylinositol 3-kinase-related kinase; ATM, ataxia telangiectasia mutated; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; BRCA2, breast cancer type 2; PTEN, phosphatase and tensin homolog; KU80, Ku autoantigen protein p80; NHEJ, non-homologous end joining; HR, homologous recombination; IR, ionizing radiation; PARP-1, poly (ADP-ribose) polymerase-1; PARP-2, poly(ADP-ribose) polymerase-2; BER, base excision repair; PI3K, phosphoinositide 3-kinase; mTOR, mammalian target of rapamycin; HDAC, histone deacetylase; Chk1, checkpoint kinase 1; γH2AX, phosphor-SER139 H2AX; 53BP1, p53-binding protein 1.

* Corresponding author.
E-mail address: seth.herzon@yale.edu (S.B. Herzon).

http://dx.doi.org/10.1016/j.bmcl.2016.04.090
0960-894X/

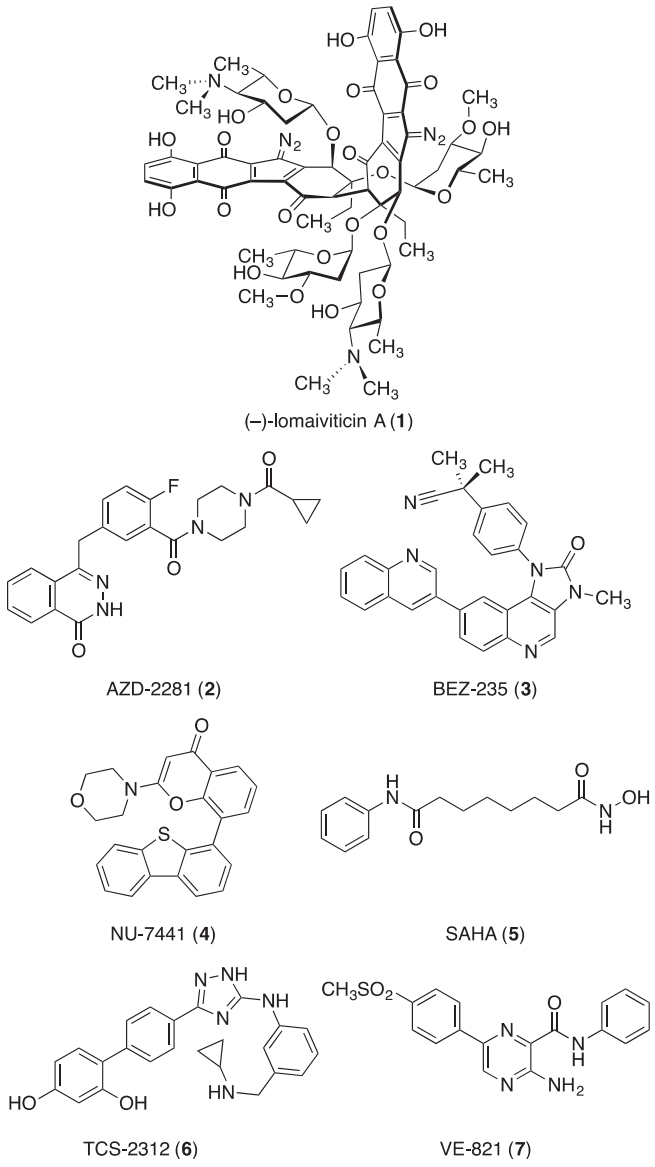

Figure 1. Chemical structures of (−)-lomaiviticin A (**1**) and the DNA repair inhibitors AZD-2281 (Olaparib, **2**), NVP-BEZ235 (Dactolisib, **3**), NU-7441 (**4**), SAHA (Vorinostat, **5**), TCS-2312 (**6**), VE-821 (**7**).

combined PI3K-mTOR inhibitor[11] and was later shown to inhibit ATM and DNA-PKcs,[12] which have catalytic domains that are homologous to PI3Ks.[13] Both DNA-PK[14] and ATM[15] are essential components of NHEJ repair. BEZ-235 (**3**) has demonstrated anticancer activity in clinical trials as well as radiosensitizing activity in preclinical models,[16] and is synthetic lethal with cancer-associated mutations through inhibition of ATR.[17] NU-7441 (**4**) is a highly selective inhibitor of DNA-PK (IC_{50} = 14 nM)[5] that causes persistence of doxorubicin- and IR-induced DNA DSBs and induces chemo- and radio-potentiation in DNA-PK-proficient (MO59-Fus-1) human tumor cells.[18] SAHA (Vorinostat, **5**) is an inhibitor of the HDAC proteins HDAC1 (IC_{50} = 10 nM) and HDAC3 (IC_{50} = 20 nM).[19] Combinations of HDAC inhibitors with radiation and chemical agents enhance cell kill as a result of chromatin relaxation mediated by HDAC inhibition, allowing increased DNA damage in transcriptionally active DNA regions.[20] **5** has also been suggested to inhibit HR repair directly.[21] TCS-2312 (**6**) is a Chk1 inhibitor (EC_{50} = 60 nM).[22] Chk1 regulates G2/M and S-phase cell-cycle checkpoints and is activated by phosphorylation in response to various types of DNA-damaging agents, including DNA-strand-breaking agents such as IR and topoisomerase inhibitors, and those agents that cause replication stress such as UV, hydroxyurea and 5-fluorouracil.[23] **6** enhances the cell killing of gemcitabine in breast and prostate cancer cell lines and displays antiproliferative activity in vitro.[22] VE-821 (**7**) is an inhibitor of ATR (IC_{50} = 26 nM) that also shows inhibition of H2AX phosphorylation.[24] **7** is highly selective for ATR (>600-fold over related kinases such as ATM or DNA-PK).[25] Inhibition of ATR by **7** is synthetic lethal with DNA-damaging agents in cancer cells deficient in ATM or p53.[24]

We employed the CellTiter-Glo luminescent cell viability assay (Promega) to identify DNA repair inhibitors that synergistically potentiate the cytotoxicity of (−)-lomaiviticin A (**1**) in K562 cells. The assay is based on the measurement of ATP using firefly luciferase and is commonly used to estimate the number of viable cells in high-throughput screening applications.[26] Cells were incubated under optimal growth conditions in 96-well plates with either a DNA repair inhibitor alone, **1** alone, or combinations of each. After 24 or 48 h of treatment, the reconstituted CellTiter-Glo enzyme–substrate mixture was added and the luminescence was measured. Synergistic potentiation of the cytotoxicity of **1** in the presence of DNA repair inhibitors was assessed using the combination index (CI) theorem of Chou–Talalay[27] where CI < 1 indicates synergism, CI = 1 indicates an additive effect, and CI > 1 indicates antagonism.

Cell viability assays conducted with BEZ-235 (**3**), SAHA (**5**), or TCS-2312 (**6**) alone indicated that cell viability decreased significantly with increasing concentrations of **3**, **5**, or **6** (0.01–2.8, 0.25–50, 0.1–20 μM, respectively, Figs. S1–S3). Accordingly, synergism studies with (−)-lomaiviticin A (**1**) were conducted at fixed ratios of **3**, **5**, or **6** to **1**. Cell viability assays conducted with AZD2281 (**2**), NU-7441 (**4**), VE-821 (**7**) revealed that cell viability did not decrease significantly with increasing concentrations of **2**, **4**, or **7** (up to 2.5–10 μM, Figs. S4–S6).

Comprehensive data depicting the results of these studies at fixed and varying ratios of (−)-lomaiviticin A (**1**) and **2**–**6**, and varying incubation periods (24 or 48 h), are presented in the Supporting information (Figs. S7–S15). Selected CI and fraction affected (Fa) values as a function of dose and incubation time are shown in Table 1. Combinations of AZD-2281 (**2**), BEZ-235 (**3**), NU-7441 (**4**), or SAHA (**5**) displayed modest synergistic effects or antagonist effects (CI = 0.81–2.1) at 0.036–0.72 fraction affected (entries 1–6). However, TCS-2312 (**6**) and VE-821 (**7**) displayed high levels of synergism with **1** (entries 7–9). Treatment of K562

Table 1
Selected values of the fraction affected (Fa) and combination indices (CI) for binary mixtures of (−)-lomaiviticin A (**1**) and the DNA repair inhibitors **2**–**7**

Entry	DDR inhibitor, dose	Dose **1** nM	*t* (h)	Fa	CI
1	AZD-2281 (**2**), 5.0 μM	5.0	24	0.036	1.2
2	AZD-2281 (**2**), 5.0 μM	5.0	48	0.56	2.1
3	BEZ-235 (**3**), 0.05 μM	0.25	48	0.61	0.81
4	NU-7441 (**4**), 5.0 μM	2.5	24	0.72	0.86
5	NU-7441 (**4**), 5.0 μM	0.50	48	0.68	1.1
6	SAHA (**5**), 0.50 μM	0.10	48	0.33	1.5
7	TCS-2312 (**6**), 0.50 μM	0.40	24	0.78	0.20
8	VE-821 (**7**), 10 μM	**0.25**	**24**	**0.65**	**0.069**
9	VE-821 (**7**), 10 μM	**0.050**	**48**	**0.60**	**0.13**