Design of Coibamide A Mimetics with Improved Cellular Bioactivity

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ABSTRACT: Coibamide A, a cyclic depsipeptide isolated from a Panamanian marine cyanobacterium, shows potent cytotoxic activity via the inhibition of the Sec61 translocon. We designed a coibamide A mimic in which the ester linkage between MeThr and d-MeAla in coibamide A was replaced with an alkyl linker to provide a stable macrocyclic scaffold possessing a MeLys(Me) residue. Taking advantage of a facile solid-phase synthetic approach, a structure–activity relationship (SAR) study of the newly designed macrocyclic structure was performed, with a focus on altering the pattern of N-methyl substitution and amino acid configurations. Overall, the simplified macrocyclic scaffold with an alkyl linker resulted in a significantly reduced cytotoxicity. Instead, more potent coibamide A derivatives with a β-(4-biphenylyl)alanine (Bph) group were identified after the optimization of the Tyr(Me) position in the original macrocyclic scaffold of coibamide A based on the characteristic apratoxin A substructures. The similar SAR between coibamide A and apratoxin A suggests that the binding site of the Tyr(Me) side chain at the luminal end of Sec61α may be shared.

KEYWORDS: apratoxin A, biphenylalanine, coibamide A, macrocyclic peptide, Sec61, translocon

Coibamide A (CbA, 1) is a highly N-methylated cyclic depsipeptide isolated from a Panamanian marine cyanobacterium ([Figure 1]).1,2 This macrocyclic natural product shows highly potent antiproliferative activity against many cell lines, with a pattern of selectivity suggestive of a distinct mechanism of action.1 In glioblastoma cells, CbA (1) induces autophagosome accumulation via a mammalian target of rapamycin (mTOR)-independent mechanism.3 The autophagy is mediated by autophagy-related protein 5 (ATG5), while CbA-induced apoptosis is independent of the presence of ATG5.4 The autophagosome clearance defects are caused by the abrogation of the autophagosome-lysosome fusion process via the impaired glycosylation of lysosomal membrane proteins LAMP1 and LAMP2.5 Cellular treatment of CbA (1) also prevents the extracellular secretion of vascular endothelial growth factor receptor 2 (VEGFR2) and epidermal growth factor receptor (EGFR) or the possible degradation via the hydrolysis of the labile ester bond of MeThr5 and carboxy group of D-MeAla11 in CbA (1) was substituted with an alkyl tether ([Figure 1]).6 The resulting arrangement of MeLys(Me) at the β-elimination of O-acyl threonine to enhance the molecular stability. Additionally, two MeSer(Me) moieties in 1 were substituted with MeAla (MeAla3 and MeAla6) because the bioactivity of the MeAla analogue was comparable to that involved in the pathological process, Sec61 is a potential molecular target for anticancer and anti-infective agents.1,12 To date, there have been several Sec61 inhibitors reported,13,14 including apratoxin A15,16 decatransin,17 eeyarestatin I,18,19 HUN-7293/pestahivin,20-22 ipomoeassin F23 and mycolactone A and B24 (Figure S1). For the application of these promising inhibitors to drug discovery, considerable efforts have been devoted to their medicinal chemistry studies.25-31 On the basis of these insights into Sec61 inhibitors, we investigated the structure–activity relationships (SARs) of CbA (1) in this study.

We designed a simplified analogue 2 in which the ester linkage between the hydroxyl group of l-MeThr3 and carboxy group of d-MeAla11 in 1 was substituted with an alkyl tether ([Figure 1]). The resulting arrangement of MeLys(Me) at the MeThr3−d-MeAla11 moiety would provide resistance against possible degradation via the hydrolysis of the labile ester bond or the β-elimination of O-acyl threonine to enhance the molecular stability. Additionally, two MeSer(Me) moieties in 1 were substituted with MeAla (MeAla3 and MeAla6) because the bioactivity of the MeAla analogue was comparable to that

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of the parent peptide, as reported previously. These modifications would facilitate the synthesis of a series of derivatives, especially to avoid the epimerization that is possible during couplings between the MeThr hydroxy group and N-methylamino acids.

Initially, we established a synthetic route to [MeAla$_3$, MeLys(Me)$_5$, MeAla$_6$]-CbA (2a, Scheme 1). The peptide sequence was assembled by Fmoc-based solid-phase peptide synthesis (Fmoc-SPPS) using the MeLeu$_{-}$-{(2-Cl)Trt} resin. 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU)/N,N-diisopropylethylamine (DIEA) was exploited for amino acid couplings onto N-methylamino acids. For protection of the ε-amino group of Lys at the ring junction, orthogonal allyloxycarbonyl (Alloc) protection was employed. After the coupling of Lys(Alloc)$_5$, followed by the deprotection of the Fmoc group, the resin was subjected to an on-resin N-methylation protocol. Briefly, after the α-amino group of Lys(Alloc)$_5$ was activated with an o-nitrobenzenesulfonyl (Ns) group, the N$_α$-methyl group was introduced by a Mitsunobu reaction. The subsequent deprotection of the Ns group afforded the MeLys(Alloc)$_5$ residue in 5. Further couplings of the depsipeptide’s N-terminal tail (Me$_2$Val$_1$−o-Hva$_2$−MeAla$_3$−MeLeu)$^a$ using HATU/DIEA provided the linear peptideyl resin. Next, we proceeded to modify the MeLys(Alloc)$_5$. N-Methylation of the MeLys ε-amino was performed by the Pd(PPh$_3$_)$_4$/PhSiH$_3$-mediated removal of the N'-Alloc group, followed by the on-resin N-methylation protocol to construct the MeLys(Me)$_5$ residue in resin 6. The coupling of Tyr(Me)$_{10}$ onto the ε-N-methylamino group of MeLys(Me)$_5$ provided the open-chain precursor 7. Cleavage from the resin 7, followed by macroyclization with EDCI/HOAt/DIEA, gave the desired cyclic peptide 2a. As such, we developed a facile solid-phase synthesis of CbA mimetics with a MeLys-(Me) moiety at the ring junction. Using a variety of commercially available materials for the components, a series of structural analogues could be obtained by the same procedure. Of note, the resulting peptide 2a exhibited submicromolar cytotoxicity against A549 cells in an MTS assay [IC$_{50}$ (2a) = 0.42 μM].

Next, we investigated ring junction SARs for these CbA mimetics. Because substitution of the ester linkage with an ethylene tether in 2a would alter the global conformations of the cyclic substructure of 1, we attempted optimization at the MeLys(Me)$_5$ moiety in 2a via modifying the tether length, the amino acid configuration, and the presence or absence of the N-methyl group. For this purpose, we substituted several lysine (Lys) and ornithine (Orn) moieties at the i-MeLys(Me)$_5$ position of 2a (Table 1). Inversion of the stereochemistry from...
2a exhibited the most potent cytotoxicity, although it was replaced with a D-amino acid (Table 2). Removal of the MeLys(Me)5 in the cyclic substructure exhibited cytotoxicities [IC50 (2a) = 8.3 μM; IC50 (2b) = 2.2 μM; IC50 (2c) = 6.4 μM] suggesting that the N-methylation induces structural organization in the N-terminal chain, which is important for biological action. Derivatives with N-methyl-deficient modifications for MeAla7, MeLeu8, or MeLys5 in the cyclic substructure exhibited cytotoxicities nine-fold or more lower compared with that of peptide 2a [IC50 (2d) = 7.5 μM; IC50 (2e) = 3.9 μM]. In contrast to the less significant Nmethyl group of MeLys(Me)5 in 2a, all Nmethyl groups on the macrocycle backbone of 2a were indispensable for its potent biological activity.

We also assessed the cytotoxicities of epimers of peptide 2a in which one of the component amino acids in the macrocycle was replaced with a D-amino acid (Table 2). Among these, the D-MeLys(Me)5 isomer 2b and D-MeLeu9 isomer 9d exhibited moderate cytotoxicities [IC50 (2b) = 8.3 μM; IC50 (9d) = 2.6 μM]. The other epimers 9a–c and 9e showed no cytotoxicity, demonstrating that the all-L-configuration in the macrocycle of 2a is necessary for potent bioactivity. Notably, the cytotoxicity may be attributable both to the binding affinity to the target(s) and the membrane permeability if the target(s) exists in an intracellular compartment, as is the case for the Sec61 translocon target of 1.8 Considering that the permeability of cyclic peptides is highly dependent on the number and position(s) of N-methyl groups and D-amino acid(s), our findings provide support that the pattern of N-methylation and the configurations of the peptide backbone in naturally occurring 1 have been optimized over the course of molecular evolution.

With the information on a favorable backbone structure in hand, we next proceeded to optimize the aromatic amino acid at Tyr(Me)10 in 2a. To gain clues for designing the peptides, we focused on a substructure in apratoxin A (Figure S1), which is also a depsipeptide inhibitor that targets Sec61α.15,16 Similar to 1, apratoxin A contains L-Tyr(Me) as the sole aromatic amino acid, which is indispensable for the bioactivity.35 In the previous SAR study, replacing L-Tyr(Me) in apratoxin A with L-β-(4-biphenylyl)alanine (Bph) led to a >100-fold increase in its cytotoxicity.37 On the basis of this insight, we pursued the development of more potent analogues by modifying Tyr(Me)10 in 2a (Table 3). For this purpose, the solid-phase synthetic protocol for 2a was fully compatible with the divergent synthesis of derivatives in which resin 6 was employed as a common substrate for further modification.
with various aromatic amino acids. First, a series of functional
groups at the para-position of the methoxy group were
investigated (10a–j). Substituting Tyr(Me) with Phe led to an
approximately 10-fold decrease in the bioactivity [IC_{50} (10a) =
4.0 μM]. Derivatives with a Phe(4-NO_{2}), Phe(4-CN), or
Tyr(t-Bu) group showed somewhat less potent cytotoxicities
than 2a [IC_{50} (10b) = 1.1 μM; IC_{50} (10d) = 1.5 μM; IC_{50} (10h) =
1.0 μM], whereas other derivatives exhibited the same
level of cytotoxicity as 2a [IC_{50} (10c) = 0.37 μM; IC_{50} (10e) =
0.38 μM; IC_{50} (10f) = 0.71 μM; IC_{50} (10g) = 0.61 μM; IC_{50} (10i) =
0.32 μM]. As expected, Bph-containing 10j exhibited a
seven-fold more potent cytotoxicity than 2a [IC_{50} (10j) =
0.060 ± 0.016 μM]. We further designed and synthesized derivatives
(10k–q) with a variable aromatic amino acid at the Tyr(Me)^{10}
position of 2a. Pyridine-containing derivatives were inactive
(10k–m). Modification with 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic, 10o), a conformationally restricted
counterpart of Phe, or MePhe also led to a loss of bioactivity
(10n), implying that N-alkylation at the Tyr(Me)^{10} position
may unfavorably alter the conformations of the macrocycle.
Interestingly, substitution with β-(1-naphthyl)alanine (1-Nal)
led to decreased cytotoxicity [IC_{50} (10p) = 4.8 μM], while that
with β-(2-naphthyl)alanine (2-Nal) resulted in a slightly
improved potency [IC_{50} (10q) = 0.28 μM]. These observations suggest that the substituent at the para-position of the aromatic ring in Tyr(Me)^{10} significantly influences the
cytotoxicity.

Considering the enhanced potency of the Bph-containing
mimetic 10j, we designed Bph-containing analogues of the
original CbA scaffold (11 and 12, Figure 2). Depsipeptides 11
and 12 were synthesized using some modifications of the
previously reported procedure^{26} (see the Supporting
Information). As expected, Bph-containing derivative 11 showed a
cytotoxicity 12-fold more potent than that of 1 [IC_{50} (11) =
0.11 nM]. Similarly, peptide 12 with MeAla\textsuperscript{3} and MeAla\textsuperscript{6}
modifications also exhibited a potency 5.6-fold greater than
that of 1 [IC_{50} (12) = 0.25 nM]. The increased cytotoxicity
obtained by replacing Tyr(Me) with Bph in CbA analogues
was consistent with the SAR of apratoxin A analogues.\textsuperscript{27} This
common SAR provides support that the binding pocket of
Tyr(Me) at the luminal end of Sec61\textsuperscript{10} would be shared
between apratoxin A and CbA.

In summary, we designed and synthesized the CbA mimetic
2a, which contains an alkyl linkage in place of the labile ester
linkage in 1. Taking advantage of the facile synthetic protocol
established using solid-phase techniques, we investigated the
SAR of macrocyclic structures of 2a. Additionally, optimization of
the aromatic amino acid in CbA was carried out based on the
reported SAR data for another Sec61 inhibitory peptide,
apratoxin A. The substitution of Tyr(Me)\textsuperscript{10} in 2a with Bph led to
significantly increased cytotoxicities, as expected. Similarly,
peptides 11 and 12 with enhanced cytotoxicities were
identified when the favorable Bph residue was applied to
Tyr(Me)\textsuperscript{10} in 1 and an analogue peptide, respectively. To the best of our knowledge, this is the first report of the identification of more potent CbA analogues. Further investigations to develop CbA mimetics with more favorable bioactivities and physicochemical properties are ongoing in our laboratory.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00591.

Experimental procedures for peptide synthesis and biological evaluations, characterization of peptides, and supporting figures (PDF)

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Notes
The authors declare no competing financial interest.

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ABBREVIATIONS

Alloc, allyloxy carbonyl; ATG5, autophagy-related protein 5; Bph, β-(4-biphenylyl)alanine; DIC, N,N'-disopropylcarbodiimide; DIEL, 1,1-dimethyl-3-(3-(dimethylamino)propyl)carbodiimide; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; EGFR, epidermal growth factor receptor; HATU, (dimethylamino)propyl)carbodiiimid e; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; SPPS, solid-phase peptide synthesis; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; Tyr(Me), O-methyltyrosine; VEGFA, vascular endothelial growth factor A; VEGFR2, vascular endothelial growth factor receptor 2

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