Medicinal Chemistry Research (2025) 34:2292–2298
https://doi.org/10.1007/s00044-025-03486-2

MEDICINAL CHEMISTRY RESEARCH

BRIEF REPORT

Synthesis of simplified didehydro-cortistatin A derivatives as anti-proliferative agents

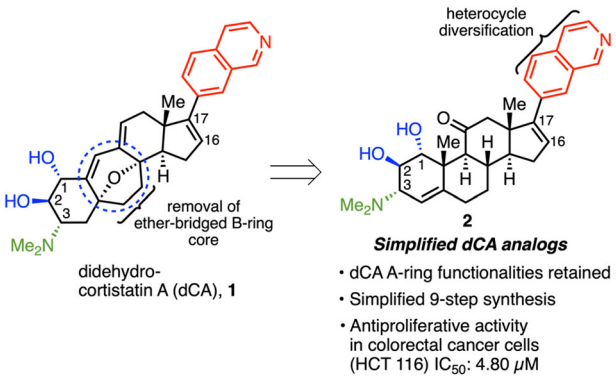
Jeremy S. Coleman[1,3] · Larissa Costa de Almeida[1] · Laura E. Hanold[1] · Michael J. Ferracane[2] · Hendrik Luesch[1] · Jane V. Aldrich[1]

Received: 31 July 2025 / Accepted: 8 October 2025 / Published online: 30 October 2025

Abstract

The natural product cortistatin A and its derivative didehydro-cortistatin A exhibit potent biological activity in different disease states, indicating the potential utility of their derivatives as treatments for a variety of diseases. The synthesis of the unique ring system found in these compounds is challenging, and therefore we designed analogs with a conventional steroidal scaffold that retained the A-ring functionalities with the stereochemistries found in the natural product, building on a previous report of simplified didehydro-cortistatin A analogs. The steroidal derivatives were synthesized in 9 steps from prednisone with different isoquinoline isomers incorporated at C17 via a Stille coupling in the last step. The analogs exhibited antiproliferative activity in HCT 116 colon cancer cells with low micromolar potency (HCT 116 $IC_{50} = 4.80–11.5\ \mu M$) and rapid onset. The methodology described here can be used to prepare additional simplified didehydro-cortistatin A analogs for future biological applications.

Graphical Abstract



Keywords Didehydro-cortistatin A analogs · Steroid derivatives · Isoquinoline isomer derivatives · Colon cancer cells

✉ Jeremy S. Coleman
Jeremy.Coleman@uga.edu

✉ Jane V. Aldrich
janealdrich@ufl.edu

[1] Department of Medicinal Chemistry, University of Florida, Gainesville, FL 32610, USA

[2] Department of Chemistry and Biochemistry, California State University Fullerton, Fullerton, CA 92831, USA

[3] Present address: Department of Pharmaceutical Sciences, University of Georgia, Athens, GA 30602, USA

Introduction

In 2006 and 2007 the Kobayashi group isolated a family of steroidal alkaloids known as cortistatins from the marine sponge *Corticium simplex* [1–4]. These structurally complex cortistatins possess a unique oxabicyclo[3.2.1]octene core centered within a 9(10–19)-*abeo*-androstane steroidal framework. Since their discovery cortistatins have garnered considerable interest across the scientific community, notably for their potent anti-angiogenic activity [1].

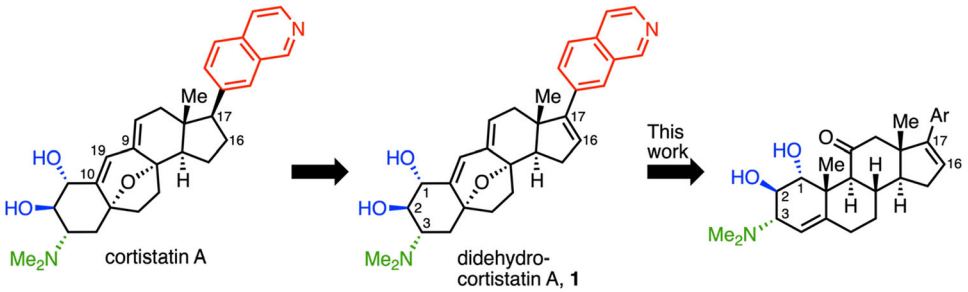


Fig. 1 Cortistatin A, didehydro-cortistatin A, and new simplified derivative scaffold

Angiogenesis, the proliferation of new capillary blood vessels from the pre-existing vasculature, aids in the progression of cancerous tumor growth, rheumatoid arthritis, psoriasis, hemangiomas, and diabetic retinopathy from ocular neovascularization [5]. Therefore, potent and selective anti-angiogenic agents possess significant therapeutic potential in treating a variety of angiogenesis-driven diseases. Notably, cortistatin A (Fig. 1), exhibited selective nanomolar antiproliferative activity against human umbilical vein endothelial cells (HUVECs, $IC_{50} = 1.8$ nM) over cancer cell lines and normal human fibroblast cells [1]. Cortistatin A also inhibits vascular endothelial growth factor-induced cellular migration and basic fibroblast growth factor-induced tubular network formation of HUVECs without observed cytotoxicity. Currently, the cellular target of cortistatin A is unknown.

To address the limited supply of this bioactive natural product for detailed biological evaluation and drug development the Baran [6], Hirama [7], Myers [8], Nicolaou-Chen [9, 10] and Shair [11] groups developed elegant synthetic strategies to obtain cortistatin A. Notably, in 2011 the Baran laboratory reported a shorter (16 step) and scalable cost-effective synthetic route to this natural product [12]. Interestingly, the penultimate compound in Baran's synthesis, didehydro-cortistatin A (dCA), **1**, containing a C16-C17 alkene, was identified as the most potent trans-activator of transcription (Tat) inhibitor known to date ($IC_{50} = 0.6$ nM), providing a novel approach to potentially treat dormant HIV infections [13]. These findings further support the utility of cortistatin A derivatives as potential therapeutics for a variety of diseases.

Given the challenges in synthesizing cortistatin A and dCA, we focused our efforts on developing simplified analogs of dCA, **1**. Historical structure-activity relationship (SAR) studies identified the C17 isoquinoline moiety as an important substituent to retain selective anti-angiogenic activity observed with cortistatin A [4]. Corey reported the synthesis of simplified dCA analogs from estrone with anti-angiogenic activity [14]; these analogs retained the C3 dimethylamine and the C17 isoquinoline, but lacked the A-ring hydroxyl groups found in cortistatin A.

Inspired by the synthetic work of both the Baran and Corey labs, we designed analogs of dCA with a conventional steroidal scaffold that retained the A-ring functionalities and their stereochemistry while exploring the SAR of the isoquinoline at the C17 position. Here we report the synthesis of three initial dCA analogs, and the evaluation of their activity in different cancer cell lines and HUVECs.

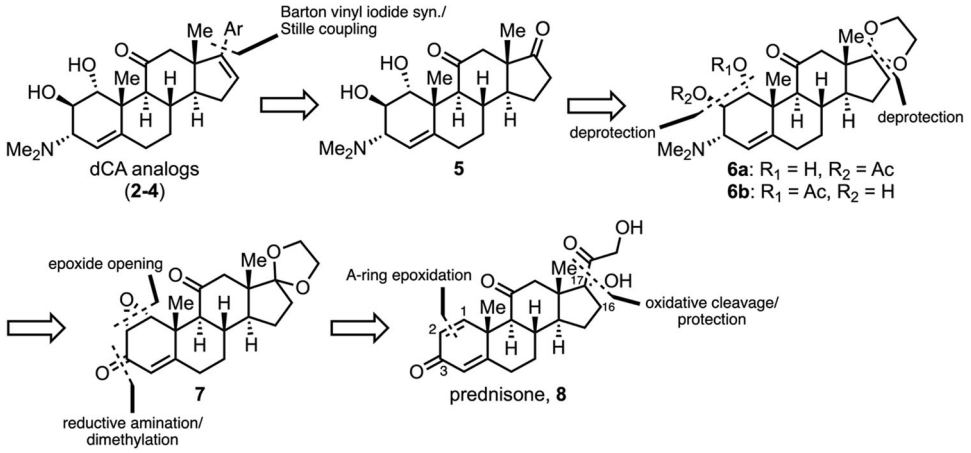
Results and discussion

Chemistry

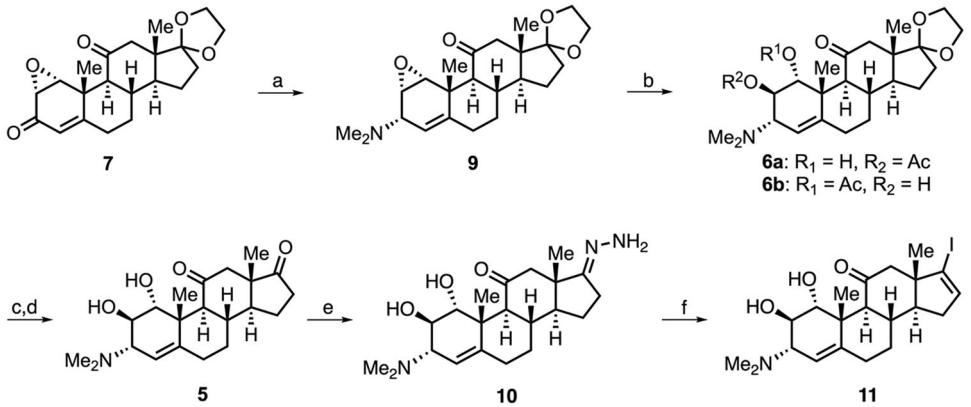
Scheme 1 shows the retrosynthetic analysis for our strategy to prepare simplified analogs of dCA. The desired analogs of dCA **2**–**4** could be generated by late-stage Stille coupling at the C17 carbonyl of precursor dione **5**. The dione can be obtained by global deprotection of a C17 ketal and a C1 or C2 acetate (regioisomers **6a** and **6b**). The acetate regioisomers can be prepared through a stereo- and regioselective reductive amination and subsequent dimethylation at C3, followed by a stereoselective epoxide ring opening of C1-C2α-epoxide **7**. The epoxide **7** is obtained by oxidative cleavage of the C17 side chain, ketal protection of the resulting ketone, and an A-ring stereoselective epoxidation starting from the commercially available anti-inflammatory drug prednisone **8**.

Following the synthesis of epoxide **7** in three steps from prednisone as described by Baran and co-workers [6], we sought a synthetic route to provide the key vinyl iodide intermediate **11** (Scheme 2). The C3 ketone was subjected to a stereo- and regioselective reductive amination to afford the C3α-dimethylamine **9** and its HCl salt. This reaction required considerable optimization. Initial attempts to prepare the dimethylamine in a single step by reductive amination of the unsaturated C3 ketone with dimethylamine using either $NaBH_3CN$ or $NaBH_4$ accompanied by the Lewis acid $Ti(O\textit{i}\text{-}Pr)_4$ were unsuccessful, which may be due to steric hindrance between the incoming amine nucleophile and the adjacent epoxide. To overcome this, a 2-step reductive amination strategy was explored with the first step being reductive amination with ammonium acetate or

Scheme 1 Retrosynthetic analysis of simplified analogs **2–4**



Scheme 2 Synthesis of precursor vinyl iodide 11



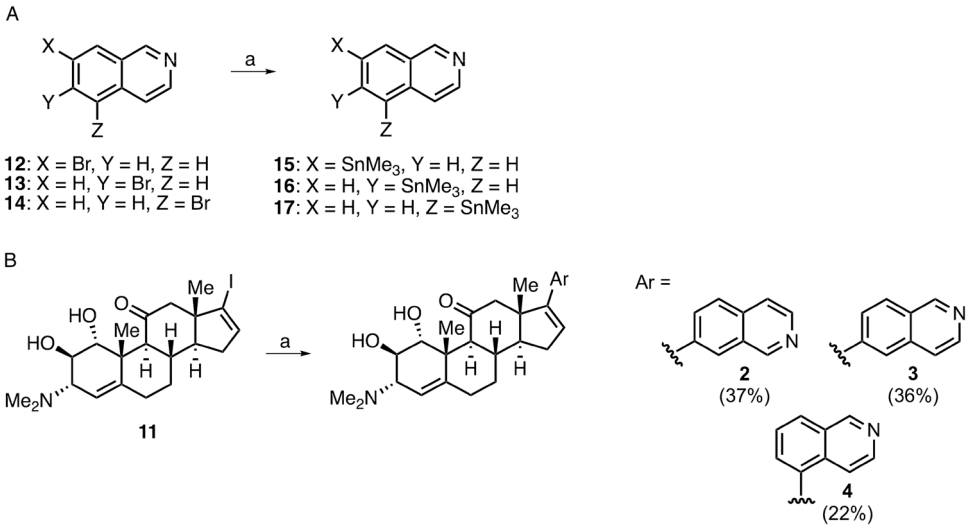
Reagents and conditions: (**a**) 1. $Ti(O\text{-}iPr)_4$, NH_3, DCM, r.t; $NaBH_4$; 2. 37% aq. CH_2O, 30% aq. AcOH, $NaBH(OAc)_3$, MeCN, rt; (**b**) AcOH, Et_3N, 170 °C, μW; (**c**) PPTS, acetone:H_2O (1:1), 90 °C; (**d**) K_2CO_3, rt; (**e**) NH_2NH_2, Et_3N, EtOH, 50 °C; (**f**) I_2, Et_3N, THF, r.t.

ammonia and a reducing agent, $NaBH_3CN$ or $NaBH_4$, respectively. Axial hydride delivery occurred exclusively from the beta face to reduce the C3 imine. Dimethylation of the resulting C3α-amine with formaldehyde then provided solely the desired C3α-dimethylamine **9**. $Ti(Oi\text{-}Pr)_4$ in the presence of ammonia and $NaBH_4$ vastly improved the yield (69% vs 25% using ammonium acetate and $NaBH_3CN$) and was readily scalable (>5 g prepared). Isolation of the HCl salt of **9** during workup allowed for full stereochemical analysis by NOE experiments. A stereoselective epoxide ring opening was then performed through acetate nucleophilic attack at C2 in the presence of triethylamine. The resulting acetate ester can undergo acyl transfer with the C1 hydroxyl group providing two inseparable regioisomers, **6a** and **6b**, in a (2:1) regioisomeric ratio. Stereochemistry is conserved in this transformation, so the mixture of regioisomers was carried forward for deprotection without separation. All stereogenic centers were confirmed by NOE analysis (see Supplementary Information).

Following installation of all A-ring substituents with their proper stereochemistry, preparation for late-stage Stille coupling commenced. Acetate deprotection of regioisomers **6a** and **6b** with K_2CO_3 gave the *trans*-vicinal diol. However, ketal deprotection using *para*-toluenesulfonic acid resulted in dehydration and the loss of either the C1 or C2 hydroxyl group. A milder transketalization procedure from the literature [15] using acetone and iodine under reflux or microwave irradiation also proved unsuccessful. A one-pot, global deprotection procedure with pyridinium *para*-toluenesulfonate (PPTS) in acetone followed by K_2CO_3 was successful, affording dione **5** in high yield (91%). Dione **5** was then converted to the C17 hydrazone **10** which underwent Barton vinyl iodide synthesis to provide the key vinyl iodide precursor **11**.

With vinyl iodide **11** containing the desired steroidal framework in hand, our focus turned to Stille coupling installation of heterocycles at C17. We selected three isoquinolines for incorporation at this position based on the

Scheme 3 Synthesis of simplified didehydro-cortistatin A analogs



Reagents and conditions: **A** (**a**) $Pd(PPh_3)_4$, hexamethylditin, LiCl, 105 °C. **B** (**a**) **15, 16**, or **17**, $Pd(PPh_3)_4$, CuCl, LiCl, DMSO, 60 °C

results reported by Corey and coworkers [14] to explore the SAR of heteroatom placement and substitution pattern of the installed isoquinoline moiety. The commercially available brominated isoquinolines **12, 13**, and **14** were treated with hexamethylditin, $Pd(PPh_3)_4$, CuCl, and LiCl to afford the corresponding trimethylstannyl-substituted heterocycles **15, 16**, and **17** in 67%, 66% and 34% yields, respectively (Scheme 3A). Subsequent Stille couplings with the prepared trimethylstannyl isoquinolines afforded the target compounds **2, 3**, and **4** (Scheme 3B).

Biological evaluation of didehydro-cortistatin A analogs

Based on the reports by Corey and Kobayashi we evaluated the newly synthesized analogs for antiproliferative activity against cancer cell lines and for anti-angiogenic activity using HUVECs. The analogs exhibited antiproliferative activity against HCT 116 colon cancer cells (Fig. 2A), with IC_{50} (and 95% confidence interval, CI) values after 48 h treatment of 4.80 (4.25–5.40), 11.5 (10.5–12.7), and 7.53 (6.25–9.04) μM for analogs **2, 3**, and **4**, respectively. The analogs produced rapid effects in these cells, with antiproliferative effects evident after treatment for only 2 h (Fig. 2B). Since the MTT assay used as a proxy for cell viability measures mitochondrial metabolic activity, we evaluated cell viability directly using the trypan blue exclusion assay following treatment of the HCT 116 cells with the analogs for 2 h (Fig. 2C). The results from the trypan blue assay were consistent with those from the MTT assay, suggesting the compounds do not simply inhibit mitochondrial activity but rapidly reduce cell viability, indicating that the cytotoxicity mechanism of the analogs is not primarily mitochondria related [16]. These compounds were also evaluated for antiproliferative activity in PC3 prostate cancer cells but were less potent, with IC_{50} values of 38–49 μM (Fig. 2D), in these cells. However, the compounds did not inhibit tube formation in a HUVEC Matrigel tube formation assay at non-cytotoxic concentrations (≤1 μM, see Supplementary Information).

Conclusions

We successfully synthesized simplified analogs of didehydro-cortistatin A that retain the A-ring functionalities and stereochemistries found in dCA in 6 steps from epoxide **5** (9 steps from prednisone). These simplified analogs retained antiproliferative activity in HCT 116 colon cancer cells with similar potencies to those reported for cortistatin A [1] and other cortistatins [4] in other cancer cell lines ($IC_{50} = 6–7$ μM). Notably, the antiproliferative activity of our steroidal derivatives in HCT 116 colon cancer cells had a rapid onset, with antiproliferative activity evident after only 2 h treatment. Interestingly, our analogs were much less potent in prostate cancer PC3 cells. Similar to our findings for the simplified dCA analogs in HCT 116 cells, in the cortistatins various heterocycle-containing substituents, in addition to the 7-isoquinoline found in cortistatin A, were tolerated at C17 with retention of antiproliferative activity; in contrast, the antiproliferative activity of these natural products was very sensitive to other D-ring substitutions [4].

However, unlike cortistatin A and the steroidal compounds reported by Corey and coworkers [14] the simplified steroidal analogs described here did not exhibit

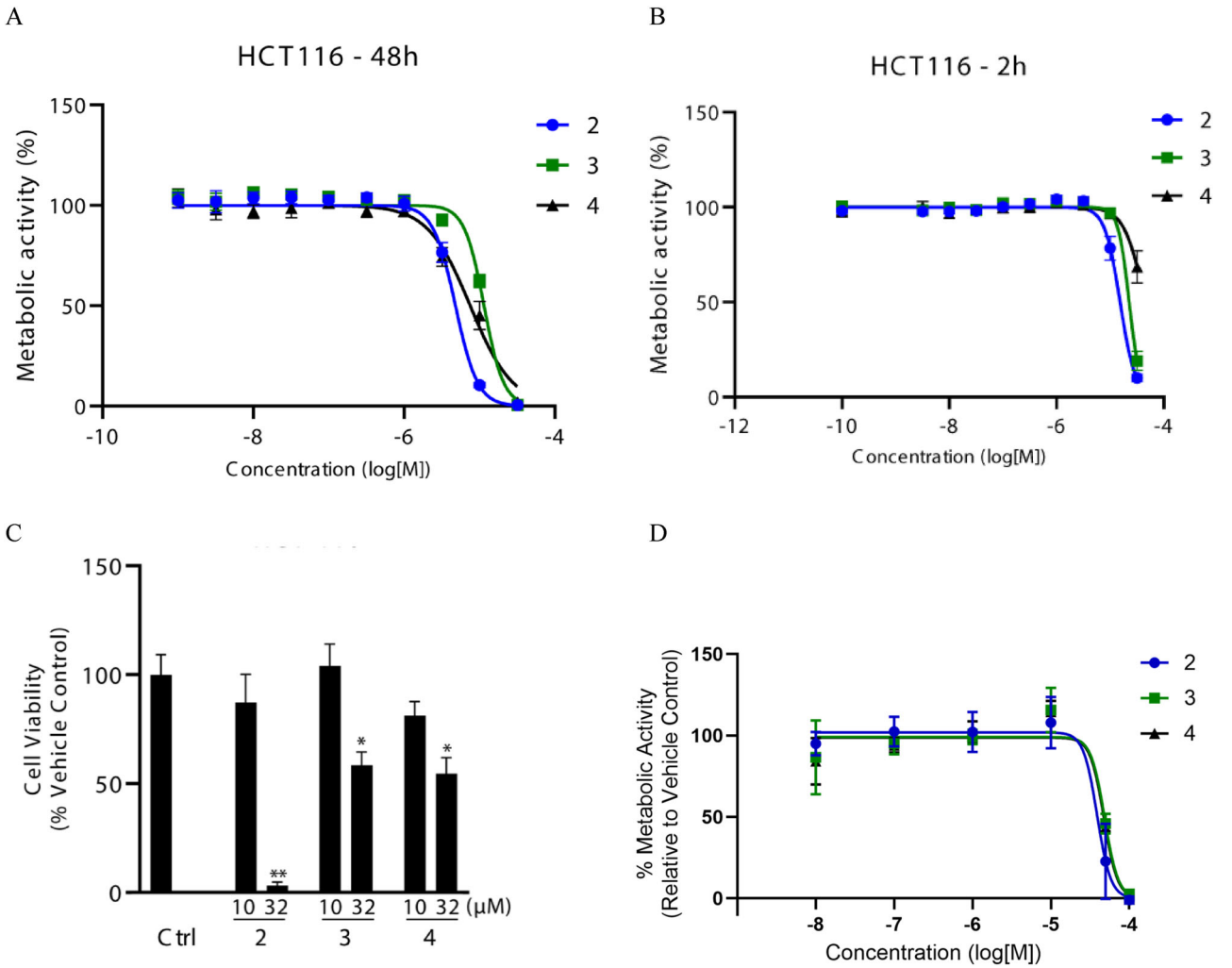


Fig. 2 Simplified dCA analogs exhibit antiproliferative activity in HCT 116 colon cancer cells after treatment for (**A**) 48 h and (**B**) 2 h based in the MTT assay which measures metabolic activity. **C** Evaluation of the effects of analog treatment of HCT 116 cells for 2 h in the trypan blue assay. **D** Antiproliferative activity of the analogs in PC3 prostate cancer cells following treatment for 48 h (WST-1 assay measuring metabolic activity)

anti-angiogenic activity at concentrations up to 1 μM. There are significant structural differences between cortistatin A with its 7-membered, ether-bridged B-ring and the compounds we and Corey et al. synthesized with a standard steroidal backbone (Fig. 3). This results in different orientations for the A ring amino and hydroxyl groups as well as the isoquinolyl group common to cortistatin A and our steroidal analogs. While our analogs and those reported by Corey and coworkers have the standard steroidal skeleton, they differ in the stereochemistry at the 3 position as well as the presence/absence of the Δ^4 double bond. These structural features have subtle effects on the conformation of the A ring and the orientation of the dimethylamine and hydroxyl groups; notably, Corey's compounds lack the hydroxyl groups in the A ring that are retained in our analogs. In addition, our analogs contain the C19 angular methyl group that is not present in either Corey's analogs or the cortistatins. These confounding factors make it challenging to deduce the structural requirements for both antiproliferative and anti-angiogenic activity across the steroidal derivatives. Still, the modeling helps identify similarities and differences between cortistatin A and the steroidal analogs which can be used with our current SAR work to inform the design of future analogs.

The results presented here support additional investigation in future studies of the biological activity of such simplified dCA analogs. This includes additional exploration of their antiproliferative activity in cancer cells. The methodology described in this report can be used to synthesize additional analogs that can also be explored for other biological activities. This includes binding to Tat where the A-ring functionalities may contribute to the biological activity of dCA [17].

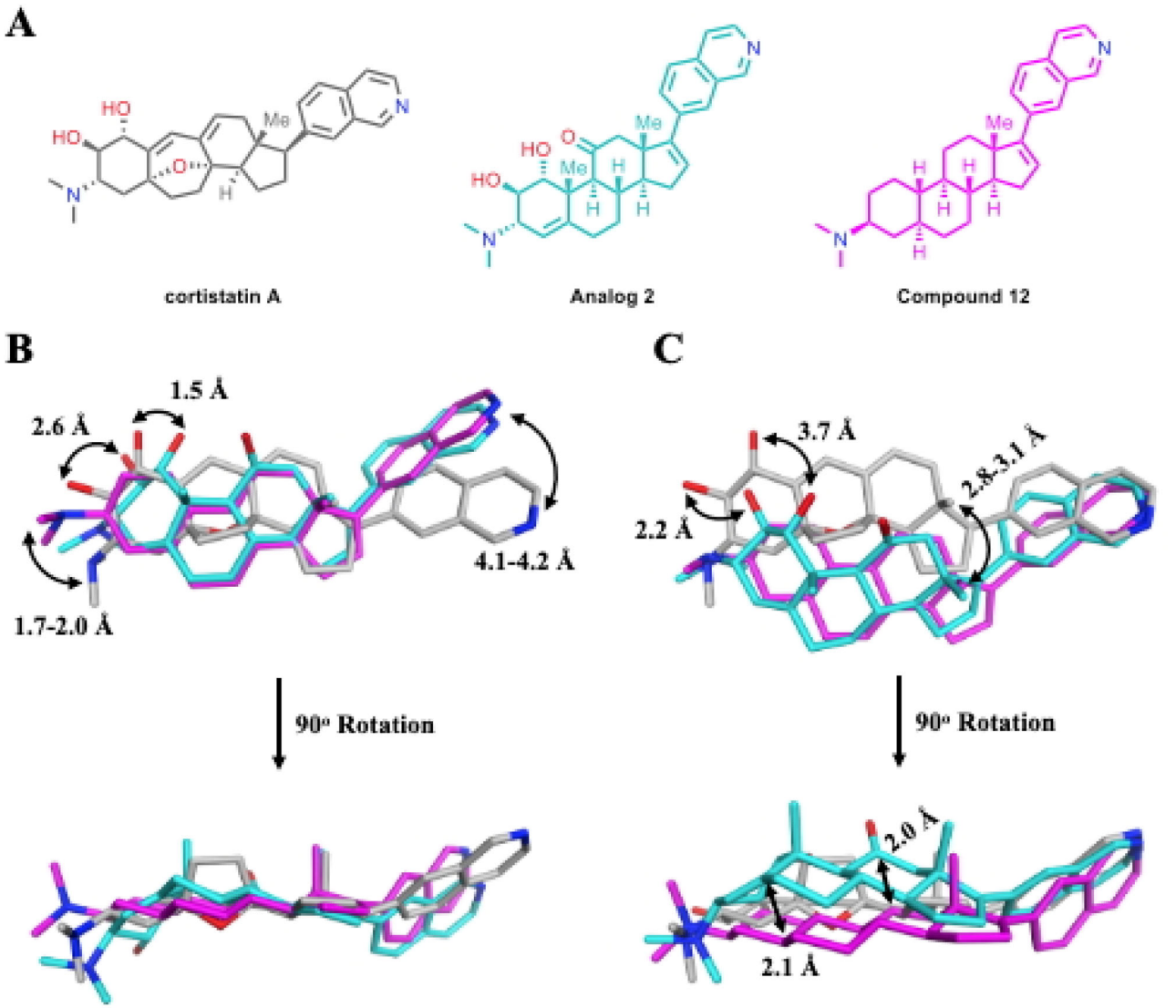


Fig. 3 Structural comparisons of cortistatin A (gray), analog **2** (cyan) described herein, and compound 12 (magenta) reported by Corey and coworkers [14]. **A** Structures of the compounds. **B** Structural overlay using the central ring systems results in poor overlap of the isoquinoline groups (4.1 and 4.2 Å between nitrogen atoms) but smaller deviations for the amine (1.7 and 2.0 Å between nitrogen atoms) and hydroxyl groups (1.5 and 2.6 Å between oxygen atoms) in the A ring. **C** Structural overlay using the C3-amine and C17-isoquinoline groups results in large differences in the central ring systems (2.8 and 3.1 Å between angular methyl carbon atoms) and the hydroxyl groups (2.2 and 3.7 Å between oxygen atoms). Due to the differences in stereochemistry and hybridization of atoms in ring A, the two steroid scaffolds also deviate from each other (2.0 and 2.1 Å between carbons 11 and 5) when overlaid in this manner

Data availability

No datasets were generated or analyzed during the current study.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s00044-025-03486-2.

Acknowledgements This research was supported by the Department of Medicinal Chemistry, NIH grant 1 R01 DA039044 to Dr. Jay McLaughlin in the College of Pharmacy, and the Debbie and Sylvia DeSantis Chair Professorship (H.L.) at the University of Florida. The authors thank Jim Rocca for his expert assistance with the NMR analysis of these compounds.

Author contributions J.S.C. performed all of the synthesis, L.C.dA. and L.E.H. performed the biological evaluation, M.J.F. performed the computational analysis, J.V.A. oversaw the synthesis and testing in PC3 cells, H.L. oversaw testing in HCT 116 and HUVEC cells, J.V.A. and J.S.C. conceived the study; all the authors wrote the manuscript and participated in its revision. All authors reviewed and approved the final manuscript.

Competing interests A patent application was submitted on the analogs described herein.

Abbreviations

dCA	didehydro-cortistatin A
HUVEC	human umbilical vein endothelial cell
PPTS	pyridinium p-toluenesulfonate
SAR	structure-activity relationship
Tat	transactivator of transcription
WST-1	water-soluble tetrazolium

References

1. Aoki S, Watanabe Y, Sanagawa M, Setiawan A, Kotoku N, Kobayashi M. Cortistatins A, B, C, and D, anti-angiogenic steroidal alkaloids, from the marine sponge corticium simplex. J Am Chem Soc. 2006;128:3148–9.
2. Aoki S, Watanabe Y, Tanabe D, Setiawan A, Arai M, Kobayashi M. Cortistatins J, K, L, novel abeo-9(10-19)-androstane-type steroidal alkaloids with isoquinoline unit, from marine sponge Corticium simplex. Tetrahedron Lett. 2007;48:4485–8.
3. Watanabe Y, Aoki S, Tanabe D, Setiawan A, Kobayashi M. Cortistatins E, F, G, and H, four novel steroidal alkaloids from marine sponge Corticium simplex. Tetrahedron. 2007;63:4074–9.
4. Aoki S, Watanabe Y, Tanabe D, Arai M, Suna H, Miyamoto K, et al. Structure-activity relationship and biological property of cortistatins, anti-angiogenic spongean steroidal alkaloids. Bioorg Med Chem. 2007;15:6758–62.
5. Folkman J. Seminars in medicine of the beth israel hospital, boston. clinical applications of research on angiogenesis. N Engl J Med. 1995;333:1757–63.
6. Shenvi RA, Guerrero CA, Shi J, Li CC, Baran PS. Synthesis of (+)-cortistatin A. J Am Chem Soc. 2008;130:7241.
7. Yamashita S, Iso K, Kitajima K, Himuro M, Hirama M. Total synthesis of cortistatins A and J. J Org Chem. 2011;76:2408–25.
8. Flyer A, Si C, Myers A. Synthesis of cortistatins A, J, K and L. Nat Chem. 2010;2:886–92.
9. Nicolaou KC, Sun YP, Peng XS, Polet D, Chen DY. Total synthesis of (+)-cortistatin A. Angew Chem Int Ed Engl. 2008;47:7310–3.
10. Nicolaou K, Peng X, Sun Y, Polet D, Zou B, Lim C, et al. Total synthesis and biological evaluation of cortistatins A and J and analogues thereof. J Am Chem Soc. 2009;131:10587–97.
11. Lee H, Nieto-Oberhuber C, Shair M. Enantioselective synthesis of (+)-Cortistatin A, a potent and selective inhibitor of endothelial cell proliferation. J Am Chem Soc. 2008;130:16864.
12. Shi J, Manolikakes G, Yeh C, Guerrero C, Shenvi R, Shigehisa H, et al. Scalable synthesis of cortistatin A and related structures. J Am Chem Soc. 2011;133:8014–27.
13. Mousseau G, Clementz MA, Bakeman WN, Nagarsheth N, Cameron M, Shi J, et al. An analog of the natural steroidal alkaloid cortistatin a potently suppresses tat-dependent HIV transcription. Cell Host Microbe. 2012;12:97–108.
14. Czakó B, Kürti L, Mammoto A, Ingber DE, Corey EJ. Discovery of potent and practical antiangiogenic agents inspired by cortistatin A. J Am Chem Soc. 2009;131:9014–9.
15. Sun J, Dong Y, Cao L, Wang X, Wang S, Hu Y. Highly efficient chemoselective deprotection of O,O-acetals and O,O-ketals catalyzed by molecular iodine in acetone. J Org Chem. 2004;69:8932–4.
16. Luo D, Ratnayake R, Atanasova KR, Paul VJ, Luesch H. Targeted and functional genomics approaches to the mechanism of action of lagunamide D, a mitochondrial cytotoxin from marine cyanobacteria. Biochem Pharm. 2023;213:115608.
17. Mediouni S, Chinthalapudi K, Ekka M, Usui I, Jablonski J, Clementz M, et al. Didehydro-cortistatin A inhibits HIV-1 by specifically binding to the unstructured basic region of tat. Mbio. 2019;10:e02662.