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Synthesis of a C₁-C₂₃ fragment of the archazolids and evidence for V-ATPase but not COX inhibitory activity.

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Abstract

A convergent synthesis of a C₁-C₂₃ fragment of the archazolids has been completed based on a high yielding Stille coupling to construct the substituted *Z,Z,E*-conjugated triene. After removal of the protecting groups, the resulting tetrol exhibited evidence for inhibition of the vacuolar-type ATPase (V-ATPase) but not cyclooxygenase (COX) inhibitory activity.

Graphical Abstract

Keywords

archazolid; natural product; Stille reaction; V-ATPase; cyclooxygenase; total synthesis

The archazolid natural products¹ (A-F, Figure 1) constitute a family of highly potent (sub-nanomolar IC₅₀) and selective vacuolar-type ATPase (V-ATPase) inhibitors that have shown promising activity against a number of particularly aggressive and lethal cancers including trastuzumab resistant breast cancer,² glioblastoma multiforme (GBM),³ and T-cell acute lymphoblastic leukemia (T-ALL).⁴ More recent studies indicate that the archazolids also block iron metabolism and thereby mediate a therapeutic effect in breast cancers,⁵ they modulate release of tumor-promoting cytokines,⁶ and when combined with the p53 activator nutlin-3a, synergistically induce tumor cell death.⁷

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Supporting Information

YES

Primary Data

NO

All members consist of a structurally similar 24-membered macrolactone and thiazole/carbamate side chain. Glycosylation at either the C₇- or C₁₅-hydroxyls (archazolids C and E respectively) significantly reduces their V-ATPase inhibitory activity,^{1d} indicating that these two groups form important interactions with the enzyme. Interestingly, these same hydroxyls are connected by a *Z,Z,E*-conjugated triene unique to the archazolids.

Several synthetic strategies have been reported for the pharmacophorically relevant conjugated triene region of the archazolids (Figure 2). Menche's group ultimately utilized a 3-step aldol condensation after attempted Horner-Wadsworth-Emmons reactions failed,⁸ which then required a late-stage enantioselective CBS-reduction to install the C₁₅ hydroxyl. Two palladium-catalyzed cross-couplings have also been described, one a successful, albeit low-yielding, Stille reaction⁹ and the other a similar yet simpler Negishi coupling.¹⁰ Recently our group reported a synthesis of the archazolid triene by cross-metathesis (CM).¹¹ While promising, the convergence of this CM strategy is limited, for example due to the requirement of using a *cis* homodimer and issues associated with metathesis back-biting.^{12c} Combined with non-ideal aspects of the other synthetic approaches (e.g. low yields or requisite late-stage manipulations), we continued to explore alternative disconnections.

Herein we report the synthesis of a C₁-C₂₃ fragment of the archazolids based on a high yielding and convergent Stille coupling for construction of the conjugated triene (Figure 3). The choice of coupling partner identity (i.e. which was the organostannane and halide) proved critical to the success of this reaction. After removal of the protecting groups, the V-ATPase and cyclooxygenase (COX) inhibitory activities of the resulting tetrol **1** were then assayed. The results suggest some level of V-ATPase inhibition by this compound but no significant interaction with COX.

When considering alternative non-metathesis based archazolid syntheses, we nonetheless wanted to make use of the chemistry developed for these prior approaches.¹² To that end, aldehyde **2** was identified as a suitable starting point (Scheme 1). This compound had been previously prepared en route to dihydroarchazolid B.^{12c} We argue that saturation of the C₂-C₃ olefin (*ref.* Figure 1) would not be expected to negatively impact archazolid biological activity, but may simplify their synthesis and improve stability. In preparation for possible palladium-catalyzed cross-couplings, aldehyde **2** was first converted to vinyl iodide **3** in 85% as an 8:1 mixture of *Z:E* isomers by ¹H NMR at the newly formed alkene.¹³

Our “western hemisphere” synthesis^{12b} was adapted to produce an appropriate vinyl stannane coupling partner, accomplished through the use of phosphonate **4** which is available in two steps from known Weinreb amide **5**¹⁴ (Scheme 2) Horner-Emmons olefination¹⁵ with aldehyde **6**¹⁶ gave ketone **7** which was then reduced and methylated as previously described.^{12b} Unfortunately all attempts to couple stannane **8** and iodide **3** failed. In each, unreacted iodide **3** was observed, suggesting difficulties in the oxidative addition step of the catalytic cycle, perhaps sterically hindered by the γ -methyl group.

Gratifyingly, switching the sense of organometallic/halide in these reactions now led to success with the couplings. Specifically, iododestannylation¹⁷ of **8** gave iodide **9**, and iodide **3** was converted to stannane **10** by lithium halogen exchange and trapping with Bu₃SnCl

(Scheme 3).¹⁸ These two compounds underwent very efficient coupling using Furstner's conditions,¹⁹ providing **11** in 82% yield.

We were intrigued about the possibility of a compound of type **11** inhibiting V-ATPase function. Others have commented on the utility of natural product-derived fragments for drug discovery,²⁰ particularly those that maintain essential pharmacophoric features. Previously we had tested both “western” (**12**) and “eastern” (**13**) hemispheres of the archazolids using an *Arabidopsis* V-ATPase assay and found that neither displayed measurable inhibitory activity (Figure 4).^{12b} However, these compounds lacked the important linked C₇- and C₁₅-hydroxyls present in tetrol **1**.

As indicated in Figure 5, compound **1**²¹ displayed dose-dependent growth inhibition of etiolated *Arabidopsis*.²² A key component in the etiolated habit is stem elongation driven by V-ATPase mediated cell expansion,²³ such that monitoring seedling stem length provides a measurement for V-ATPase activity. Previously we demonstrated that stem elongation in *Arabidopsis* seedlings is inhibited by known V-ATPase inhibitors concanamycin A²⁴ and bafilomycin,²³ with concanamycin A exhibiting four times the potency of bafilomycin.^{12b} The IC₅₀ value for compound **1** in this assay was approximately two orders of magnitude greater than that of concanamycin A and thus also the archazolids.²⁵ Nonetheless, this modest activity is significant given the major structural differences and overall simplification of **1** relative to the natural product.

Based on a recent report by Reker et al.,²⁶ we may also suspect other biochemical targets for compound **1**. In their study, archazolid A (**ArcA**) was dissected into four hypothetical fragments (e.g. **ArcA-1**, Figure 6) from which potential targets were predicted. This computational exercise identified primarily proteins associated with arachidonic acid (e.g. cyclooxygenase, COX). However when tested, the COX-2 inhibitory activity of **ArcA** was weak (24 ± 6% inhibition at 10 μM). Reker et al. suggest this disconnect between predicted hypothetical fragment activities and the actual natural product could be due to the COX active site being buried, allowing for binding of smaller fragments (e.g. **ArcA-1** and arachidonic acid) but not **ArcA**.²⁶

We saw compound **1** as an opportunity to add additional experimental data to this theoretical work, representing a fragment similar to the hypothetical fragment **ArcA-1**. Interestingly, compound **1** did not show a dose response of greater than 5% inhibition of COX-1 or COX-2 at concentrations between 1 and 200 μM.²⁷ It is possible that compound **1** (C₂₃) is similarly too large compared to **ArcA-1** (C₁₇) and arachidonic acid (C₂₀) to bind COX. Alternatively, the inactivity of **1** could suggest that a carboxylic acid terminus is critical, which is known to be important for other COX inhibitors.²⁸ However, this would not explain the measureable activity of **ArcA** which also lacks a carboxylic acid. Other factors such as binding entropies²⁹ might also therefore need to be considered (with **ArcA** being more conformationally restricted than **1**) to understand the differential COX-inhibitory activity of these compounds.

In summary, we have developed an efficient synthesis of the archazolid macrolactone (C₁-C₂₃) framework. The resulting fragment **1** displayed evidence for inhibition of the V-

ATPase, in line with the importance of properly linked C₇ and C₁₅-hydroxyls for archazolid/V-ATPase binding. Compound **1** was also assayed for COX inhibition based on previously reported predicted activities for a structurally similar hypothetical fragment. The results showed no significant COX inhibitory activity for **1** (<5% inhibition at concentrations up to 200 μM) suggesting certain structural requirements for COX binding (i.e. carboxylic acid or macrocycle). Current efforts are aimed at advancing our understanding of archazolid structure-activity-relationships,³⁰ by utilizing **1** as a starting point to further probe the archazolid/V-ATPase and archazolid/COX interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

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References and Notes

- (1). (a)Sasse F; Steinmetz H; Höfle G; Reichenbach H Archazolids, J. Antibiot 2003, 56, 520.
(b)Menche D; Hassfeld J; Steinmetz H; Huss M; Wieczorek H; Sasse F Eur. J. Org. Chem 2007, 1196.(c)Menche D; Hassfeld J; Steinmetz H; Huss M; Wieczorek H; Sasse FJ Antibiot 2007, 60, 328.(d)Horstmann N; Essig S; Bockelmann S; Wieczorek H; Huss M; Sasse F; Menche DJ Nat. Prod 2011, 74, 1100.
- (2). von Schwarzenberg K; Lajtos T; Simon L; Mueller R; Vereb G; Vollmar AM Mol. Onc 2014, 8, 9.
- (3). Hamm R; Zeino M; Frewert S; Efferth T Toxicol. App. Pharm 2014, 281, 78.
- (4). Zhang S; Schneider LS; Vick B; Grunert M; Jeremias I; Menche D; Müller R; Vollmar AM; Liebl J Oncotarget 2015, 6, 43508. [PubMed: 26496038]
- (5). Schneider LS; von Schwarzenberg K; Lehr T; Ulrich M; Kubisch-Dohmen R; Liebl J; Trauner D; Menche D; Vollmar AM Cancer Res 2015, 75, 2863. [PubMed: 26018087]
- (6). Scherer O; Steinmetz H; Kaether C; Weinigel C; Barz D; Kleinert H; Menche D; Müller R; Pergola C; Werz O Biochem. Pharmacol 2014, 91, 490. [PubMed: 25107704]
- (7). Schneider LS; Ulrich M; Lehr T; Menche D; Muller R; von Schwarzenberg K Mol. Oncol 2016, 10, 1054. [PubMed: 27157929]
- (8). (a)Menche D; Hassfeld J; Li J; Rudolph SJ Am. Chem. Soc 2007, 129, 6100.(b)Menche D; Hassfeld J; Li J; Mayer K; Rudolph S J. Org. Chem 2009, 74, 7220. [PubMed: 19739663]
- (9). Roethle PA; Ingrid TC; Trauner DJ Am. Chem. Soc 2007, 129, 8960.
- (10). Huang Z; Negishi E-i. J. Am. Chem. Soc 2007, 129, 14788. [PubMed: 17985915]
- (11). Swick SM; Schaefer SL; O'Neil GW Tetrahedron Lett 2015, 56, 4039. [PubMed: 26257444]
- (12). (a)O'Neil GW; Black MJ Synlett 2010, 107.(b)Tran AB; Melly G; Doucette R; Ashcraft B; Sebren L; Young J; O'Neil GW Org. Biomol. Chem 2011, 9, 7671. [PubMed: 21931924] (c)King BR; Swick SM; Schaefer SL; Welch JR; Hunter EF; O'Neil GW Synthesis 2014, 46, 2927. [PubMed: 31543555]
- (13). Loiseleur O; Koch G; Cercus J; Schürch F Org. Proc. Res. Dev 2005, 9, 259.
- (14). Drouet KE; Theodorakis EA J. Am. Chem. Soc 1999, 121, 456.
- (15). (a)Sinisterra JV; Mouloungui Z; Delmas M; Gaset A Synthesis 1985, 1097.(b)Paterson I; Yeung K-S; Smaill JB Synlett 1993, 774.
- (16). Mandal AK; Schneekloth JS; Kuramochi K; Crews CM Org. Lett 2006, 8, 427. [PubMed: 16435851]
- (17). Boerding S; Bach T Chem. Comm 2014, 50, 4901. [PubMed: 24691561]

- (18). (a) Dineen TA; Roush WR *Org. Lett.* 2004, 6, 1523. [PubMed: 15128226] (b) The Z,Z-stereochemistry of the resulting stannane was confirmed by detailed NMR analysis. See Supplementary Information.
- (19). Fürstner A; Funel J-A; Tremblay M; Bouchez LC; Nevado C; Waser M; Ackerstaff J; Stimson C *Chem. Comm* 2008, 2873. [PubMed: 18566709]
- (20). Crane EA; Gademann K *Angew. Chem. Int. Ed* 2016, 55, 2.
- (21). **Compound 11**. To a solution of **9** (36 mg, 0.058 mmol, 1.0 equiv.) and **10** (45 mg, 0.058 mmol, 1.0 equiv.) in degassed THF (1.2 mL) was added [Ph₂PO₂][NBu₄] (75 mg, 0.17 mmol, 3.0 equiv.), CuTC (28 mg, 0.07 mmol, 2.5 equiv.) and Pd(PPh₃)₄ (7 mg, 0.006 mmol, 0.1 equiv.) and the mixture was stirred for 15 h. The reaction was quenched with aq. NaHCO₃ (15 mL) and extracted with MTBE (2 × 15 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated *in vacuo*. Purification by flash chromatography on silica (20:1 to 10:1 hexanes:ethyl acetate) gave **11** (46 mg, 82%) as an oil. $[\alpha]_D^{20} = -5.2$ (c 0.5, CH₂Cl₂). IR (ATR) 3062, 2983, 1736, 1614, 1415, 1274, 1267, 1129, 1078, 930 cm⁻¹. ¹H NMR (500 MHz, C₆D₆): δ 6.80 (d, *J* = 16.0 Hz, 1H), 6.44 (ddd, *J* = 15.3, 10.7, 0.9 Hz, 1H), 6.17 (d, *J* = 10.4 Hz, 1H), 6.02 (s, 1H), 5.90 (dd, *J* = 16.0, 7.2 Hz, 1H), 5.58 (dd, *J* = 15.4, 8.0 Hz, 1H), 5.34 (dd, *J* = 8.4, 0.8 Hz, 1H), 5.29 (ddd, *J* = 9.8, 2.0, 1.0 Hz, 1H), 5.07 (d, *J* = 7.4 Hz, 1H), 4.29 (dd, *J* = 9.0, 6.0 Hz, 1H), 3.63 (d, *J* = 10.0 Hz, 1H), 3.58 – 3.53 (m, 2H), 3.47 (dd, *J* = 9.7, 6.2 Hz, 1H), 3.38 (dd, *J* = 9.7, 6.8 Hz, 1H), 3.18 (s, 3H), 2.71 (m, 1H), 2.42 (m, 1H), 1.96 (m, 2H), 1.91 (s, 3H), 1.86 (m, 1H), 1.89 (d, *J* = 1.0 Hz, 3H), 1.71 (d, *J* = 1.0 Hz, 3H), 1.59 (s, 3H), 1.50 (m, 2H), 1.39 (m, 2H), 1.08 (s, 6H), 1.03 (s, 6H), 1.02 (s, 3H), 1.01 (s, 6H), 1.00 (s, 3H), 0.99 (d, *J* = 7.0 Hz, 3H), 0.98 (s, 3H), 0.96 (d, *J* = 7.0 Hz, 3H), 0.95 (s, 6H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.93 (s, 3H), 0.19 (s, 3H), 0.17 (s, 3H), 0.13 (s, 6H), 0.08 (s, 6H), 0.05 (s, 6H). ¹³C NMR (125 MHz, C₆D₆): δ 137.8, 136.0, 134.9, 134.7, 133.4, 133.3, 133.0, 132.5, 130.9, 130.3, 126.3, 125.4, 73.8, 72.9, 68.5, 63.4, 63.4, 56.0, 43.7, 41.5, 40.6, 40.1, 33.1, 31.4, 30.3, 28.5, 26.7, 26.6, 26.5, 25.4, 24.8, 24.8, 24.0, 21.0, 19.1, 18.9, 18.8, 17.4, 17.2, 17.1, 16.2, 14.3, 11.2, 9.8, 8.7, -3.0, -3.6, -4.2, -4.3, -4.4, -4.7, -4.8, -4.9. HRMS (ESI⁺): Calcd for C₅₅H₁₀₈O₅Si₄ Na⁺ (M + Na)⁺: 983.7172. Found 983.7179. **Compound 1**. To a solution of **11** (25 mg, 0.026 mmol) in THF (1.2 mL) at 0 °C was added pyridine (0.3 mL) and HF•pyr. (60% HF, 0.2 mL) and the mixture was allowed to slowly warm to room temperature for 42 h. The reaction was cooled to 0 °C, diluted with ethyl acetate (15 mL) and quenched with aq. NaHCO₃ (15 mL). The layers were separated and the aqueous phase was re-extracted with ethyl acetate (2 × 15 mL). The combined organic extracts were dried over MgSO₄, filtered, and concentrated *in vacuo*. Purification by flash chromatography on silica (1:1 to 1:2 to 0:1 hexanes:ethyl acetate) gave **1** (7 mg, 58%) as an oil. $[\alpha]_D^{20} = -6.0$ (c 0.5, CH₂Cl₂). IR (ATR) 3350, 3955, 2929, 2872, 1716, 1688, 1525, 1471, 1418, 1369, 1244, 1126, 1084, 1008, 963, 919, 828, 730 cm⁻¹. ¹H NMR (500 MHz, CD₃OD): δ 6.52 (dd, *J* = 16.0, 0.7 Hz, 1H, (H13)), 6.29 (ddd, *J* = 15.0, 10.8, 0.7 Hz, 1H, (H20)), 5.90 (d, *J* = 11.0 Hz, 1H, (H19)), 5.74 (s, 1H, (H11)), 5.70 (dd, *J* = 15.8, 5.9 Hz, 1H, (H14)), 5.57 (dd, *J* = 15.2, 7.6 Hz, 1H, (H21)), 5.09 (d, *J* = 9.0 Hz, 1H, (H9)), 5.07 (d, *J* = 8.0, 1.0 Hz, 1H, (H6)), 4.46 (dd, *J* = 6.0, 3.5 Hz, 1H (H15)), 4.05 (dd, *J* = 9.0, 6.1 Hz, 1H, (H7)), 3.48 (t, *J* = 6.0 Hz, 2H, (H1a/b)), 3.38 (d, *J* = 8.4 Hz, 1H, (H17)), 3.37 (dd, *J* = 10.6, 5.5 Hz, 1H, H23a)), 3.33 (dd, *J* = 10.6, 6.5 Hz, 1H, H23b)), 3.09 (s, 3H, (OMe)), 2.36 – 2.28 (m, 2H, (H8/22)), 1.97 (t, *J* = 7.3 Hz, 2H, (H4a/b)), 1.81 (d, *J* = 1.2 Hz, 3H, (Me10)), 1.70 (s, 3H, (Me12)), 1.64 (m, 1H, (H16)) 1.58 (d, *J* = 1.0 Hz, 3H, (Me5)), 1.56 (d, *J* = 1.0 Hz, 3H, (Me18)), 1.48 – 1.40 (m, 4H, (H2/3)), 0.96 (d, *J* = 7.0 Hz, 3H, (Me22)), 0.81 (d, *J* = 7.0 Hz, 3H, (Me8)), 0.59 (d, *J* = 7.0 Hz, 3H, (Me16)). ¹³C NMR (125 MHz, CD₃OD): δ 139.3, 138.7, 134.7, 134.4, 133.6, 132.7, 131.5, 130.5, 129.9, 127.3, 126.9, 90.4, 73.1, 73.0, 71.7, 68.2, 63.0, 56.3, 42.8, 41.3, 41.2, 40.7, 33.3, 25.3, 25.0, 20.5, 17.2, 17.0, 16.3, 11.1, 10.7. HRMS (ESI⁺): Calcd for C₃₁H₅₂O₅Na⁺ (M + Na)⁺: 527.3712. Found 527.3729.
- (22). Assays were conducted side-by-side for compound **1** and concanamycin at concentrations of 0.125, 0.25, 0.5, 1.0, 2.0, 10.0, and 20.0 μM. See Supplementary Information for details.
- (23). Smart LB; Vojdani F; Maeshima M; Wilkins TA *Plant Physiol* 1998, 116, 1539. [PubMed: 9536073]
- (24). Von der Fecht-Bartenbach J; Bogner M; Krebs M; Stierhof Y-D; Schumacher K; Ludwig U *Plant Journal* 2007 50, 466. [PubMed: 17376158]

- (25). Due to short supply, archazolids themselves were not assayed. However, reported V-ATPase inhibitory activity of concanamycin A and archazolid A are similar. The IC₅₀ values for concanamycin A and archazolid A of purified *M. sexta* holoenzyme were reported as 0.8 nmol/mg V-ATPase and 0.5 nmol/mg V-ATPase respectively. See archazolid: Huss M; Sasse F; Kunze B; Jansen R; Steinmetz H; Ingenhorst G; Zeeck A; Wiczorek H BMC Biochem 2005, 6, 13. [PubMed: 16080788] and concanamycin: Huss M; Ingenhorst G; König S; Gaßel M; Dröse S; Zeeck A; Altendorf K; Wiczorek H J. Biol. Chem 2002, 277, 40544. [PubMed: 12186879]
- (26). Reker D; Perna AM; Rodrigues T; Schneider P; Reutlinger M; Mönch B; Koeberle A; Lamers C; Gabler M; Steinmetz H; Müller R; Schubert-Zsilavecz M; Werz O; Schneider G Nature Chem 2014, 6, 1072. [PubMed: 25411885]
- (27). A COX activity assay kit was purchased from Cayman Chemical (<https://www.caymanchem.com/product/760151>, accessed 10/5/2016). See Supplementary Information for details.
- (28). Blobaum AL; Marnett LJ J. Med. Chem 2007, 50, 1425. [PubMed: 17341061]
- (29). Chang C-e. A.; Chen W; Gilson MK Proc. Natl. Acad. Sci 2007, 104, 1534. [PubMed: 17242351]
- (30). (a) Bockelmann S; Menche D; Rudolph S; Bender T; Grond S; von Zezschwitz P; Muench SP; Wiczorek H; Huss MJ Biol. Chem 2010, 285, 38304. (b) Dreisigacker S; Latek D; Bockelmann S; Huss M; Wiczorek H; Filipek S; Gohlke H; Menche D; Carlomagno TJ Chem. Inf. Model 2012, 52, 2265. (c) Menche D; Hassfeld J; Sasse F; Huss M; Wiczorek H Bioorg. Med. Chem. Lett 2007, 17, 1732. [PubMed: 17239591]

Figure 1.
Structure of the archazolid natural products.

Figure 2.
Reported synthetic approaches to the archazolid conjugated triene.

Figure 3.
Novel Stille coupling-based synthesis of the archazolid triene-containing compound **1**.

Figure 4. Synthetic archazolid fragments. Only compound 1 bears the linked C₇- and C₁₅-hydroxyls known to be important for V-ATPase inhibitory activity.

Figure 5.
Select *Arabidopsis* V-ATPase assay results.²²

Figure 6. Structures of synthetic archazolid fragment 1, a hypothetical archazolid fragment ArcA-1 used by Reker,²⁶ archazolid A (ArcA), and arachidonic acid.

Scheme 1.
Vinyl iodide synthesis for cross-coupling.

Scheme 2.
Vinyl stannane synthesis and first attempts at Stille coupling.

Scheme 3.

Reagents and conditions: ((A) I₂, DCM, -10 °C, 70%; (B) ^tBuLi then Bu₃SnCl, 90%; (C) **10** (1.0 equiv.), Pd(PPh₃)₄ (5 mol%), CuTC (2.0 equiv.), [Ph₂PO₂][NBu₄] (3.0 equiv.), THF, 15 h, 82%.