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Award Number: W81XWH-10-1-0990

TITLE: Largazole as a novel and selective anti-breast cancer agent

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REPORT DATE: October 2012

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE October 2012		2. REPORT TYPE Final		3. DATES COVERED 17 September 2010 – 16 September 2012	
4. TITLE AND SUBTITLE Largazole as a novel and selective anti-breast cancer agent				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-10-1-0990	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Andrew Phillips E-Mail: andrew.phillips@yale.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Yale Univesrity New Haven, CT 06511-6614				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Breast cancer is the second most common type of cancer in the world and second most common cause of deaths in US. There is great a demand for new, small molecule, drugs that can selectively eliminate breast cancer cells. Many natural compounds have anti-tumor activities (e.g. Taxol®). Recently we achieved a total synthesis of Largazole and demonstrated that this natural compound has remarkable selectivity toward breast cancer cells. We also find that Largazole can block two cellular activities frequently associated with aggressive tumor cells. In this proposal, we will test whether dual inhibition of two oncogenic pathways may be the reason why Largazole is highly selective against tumor cells but not normal cells. We will develop more potent and selective small molecules to validate the concept that dual specificity inhibitors are better anti-cancer drugs. Our studies are expected to provide novel ideas for designing more effective therapeutics for breast cancer treatment.					
15. SUBJECT TERMS Largazole, ubiquitin E1, Histone deacetylase, inhibitor, breast cancer,					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
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1. INTRODUCTION

Histone deacetylases (HDACs) and histone acetylases (HATs) are key players in regulating transcription and histone homeostasis(8). Transcription of tumor suppressor proteins is frequently silenced in tumor cells due the hyper- or aberrant activity of HDACs. Accordingly inhibiting histone deacetylation may re-activate inappropriately silenced genes and may be able to “reverse” malignant changes(2). Inhibitors of histone deacetylase enzymes (HDACi) have recently attracted substantial attention as potential anti-cancer drugs. The selective degradation of many regulatory proteins in eukaryotic cells is mediated by the ubiquitin system(5). Proteins targeted for degradation are usually covalently ligated to a polyubiquitin chain and subsequently eliminated by the 26S proteasome. Ubiquitination of proteins is carried out by a multi-enzyme complex consisting of E1 (ubiquitin activating enzyme), E2 (ubiquitin conjugating enzyme) and E3 (ubiquitin ligase) (5). The final product of this reaction is formation of a polyubiquitinated protein with attachment of an ubiquitination through an isopeptide bond to an epsilon-amino group of certain Lys residues in the interior of the substrate. There is only one ubiquitin E1 enzyme, more than fifty ubiquitin E2 and perhaps thousands of E3 enzymes in human genome. E3 often controls the specificity and timing of substrate ubiquitination (5). Both HDAC inhibitors and ubiquitin-proteasome inhibitors have found applications in treating specific type of human tumors. However, either type of inhibitor alone does not appear to exhibit a broad spectrum of inhibition in treating a variety of human cancers. These observations have prompted investigations using a combination of both types of inhibitors in anti-tumor studies. It was found that bortezomib killed multiple myeloma cells more efficiently when combined with histone deacetylase inhibitors(11). Thus, administering two inhibitors simultaneously targeting both pathways could be a feasible therapeutic strategy for cancer treatment.

The goal of this study is to test that dual-specificity small molecules capable of targeting two or more aberrant signaling pathways associated with human cancers will be more efficacious in suppressing human tumors. We found that Largazole, a cyclic depsipeptide natural product isolated from the marine cyanobacterium by Luesch and coworkers (15), has remarkable potency in selectively inhibiting the proliferation of breast cancer cells without significant effects on normal breast mammary epithelial cells. We found Largazole also inhibits ubiquitin E1. The inhibitory activity of these small molecules on ubiquitin conjugation has been traced to their inhibition of the ubiquitin E1 enzyme. To further dissect the mechanism of E1 inhibition, we analyzed the effects of these inhibitors on each of the two steps of E1 activation. We show that Largazole and its derivatives specifically inhibit the adenylation step of the E1 reaction while having no effect on thioester formation. E1 inhibition appears to be specific to human E1 as Largazole ketone fails to inhibit the activation of Uba1p, a homolog of E1 in *Schizosaccharomyces pombe*. Moreover, Largazole analogs do not significantly inhibit SUMO E1. Thus, Largazole and select analogues are novel classes of ubiquitin E1 inhibitors and valuable tools for studying ubiquitination in vitro. This class of compounds could be further developed and potentially be a useful tool in cells. Using breast cancer 3D culture model and xenograft models, we show that Largazole and our newly made Paragazole have potent antitumor activity toward triple negative breast cancer. Our ultimate

goal is to demonstrate that dual targeting of both pathways is the underlying mechanism for the potency and selectivity of Largazole for breast cancer cells.

2. BODY---Studies and Results

Three specific aims were proposed in the original application. We describe our progress in the context of approved SOW.

Aim 1. Synthesize derivatives of largazole with potentially improved molecular properties and improved selectivity for transformed vs non-transformed cells.

- Task 1.1 Synthesize initial round of largazole analogs for SAR testing **(Phillips, Months 1-6) (Completed)**
- Task 1.2 After initial SAR data is obtained, design and synthesize a second round of focused largazole analogs **(Phillips, Months 7-12) (Completed)**
- Task 1.3 Write and submit manuscripts describing the initial phase of SAR studies as well as annual report to CDMRP **(Liu and Phillips, Month 12) (Completed)**
- Task 1.4 Synthesize larger quantities of selected largazole derivatives for detailed testing **(Phillips, Months 14-16) (started and ongoing)**
- Task 1.5 Synthesize largazole derivatives targeted to specific cancer cells e.g. folic acid derivatives **(Phillips, Months 17-23) (to be started)**
- Task 1.6 Testing the activity of largazole derivatives using HDAC1 enzymatic assay, p27 ubiquitination and E1 thiolester assays **(Liu, partially completed and ongoing).**

Results

Largazole stabilizes GFP-p27 expression in Kip16 cells. A hallmark of many advanced cancers is an excessive degradation of the cyclin-dependent kinase inhibitor p27, which is directed by SCF^{Skp2}-mediated ubiquitination. Hence, stabilization of p27 degradation represents a rational approach in cancer therapeutics. To identify small molecule inhibitors that can stabilize p27Kip1, we performed a screen of ~3000 compounds from NCI DTP diversity set along with several natural products in our collection. For the cell based screen, we generated a mink lung epithelial cell line (Kip16) stably expressing p27 that was cloned in frame with green fluorescent protein (GFP). The resulting N-terminal GFP-p27 fusion, detectable by fluorescence microscopy, was used to determine the levels of p27 expression upon treatment of cells with the compound libraries in 96-well format. Much to our surprise, the most potent hit that emerged from this screen was the natural compound Largazole (Figure 1), which was first described by Luesch and coworkers (15) and subsequently synthesized in several laboratories including ours (1, 3, 10, 14, 15, 18, 19). Largazole induced a robust and highly uniform upregulation of GFP-p27 at concentrations as low as 1 nM (Figure 2a) as compared to the expression levels after treatment with the proteasome inhibitor MG132. We did not observe an increase in GFP-p27 expression upon treatment with the vehicle control DMSO. This result suggests that Largazole can stabilize GFP-p27 expression in cultured cells.

Largazole and select analogues inhibit the *in vitro* ubiquitination of p27 and Trf1. Initial investigation into the mechanism of Largazole indicated that the compound stabilized the expression of p27 in cells. Since the concentration of cyclin-dependent kinase inhibitor p27 is mainly regulated at the protein level by increased polyubiquitination and subsequent proteasomal degradation, we hypothesized that Largazole and synthetic analogues stabilize p27 by inhibiting the ubiquitination pathway (9, 13). One of the downsides of cell based assays is that the effects observed may be attributed to the influence of multiple pathways. For example, inhibiting the proteasome, elevating transcription of GFP-p27, or inhibiting Cdk activity can also lead to an increase in p27 expression. To tease out the mechanism and action of Largazole on p27

stabilization, we decided to test the effect of Largazole on p27 ubiquitination in a fully reconstituted system *in vitro* (16, 17). To test if Largazole affects p27 ubiquitination *in vitro*, we incubated Largazole (L) with p27, ubiquitin E1, E2, SCF^{Skp2}, and Cks1. As shown in Figure 1, adding Largazole significantly reduced polyubiquitinated p27, suggesting that Largazole can block p27 ubiquitination. Since Largazole is known to be a histone deacetylase inhibitor and has a thioester moiety that links an aliphatic chain to the core, we decided to test whether inhibition of p27 degradation can be linked to its histone deacetylase inhibitory activity. The structure-activity relationship for Largazole is relatively well understood (12). Therefore we next tested a series of Largazole analogues to get a preliminary structure-activity relationship on p27 ubiquitination. To investigate this, Largazole ester (E), Largazole ketone (K), Largazole macrocycle (M), and *seco*-Largazole (S) were tested in an *in vitro* p27 ubiquitination assay (Figure 2b). We also added the HDAC inhibitor Trichostatin A (TSA), the structure of which can be found in Figure 1, to the assay to determine whether or not other HDAC inhibitors affect p27 ubiquitination. We observed that Largazole (L), Largazole ketone (K), and Largazole ester (E) inhibited the ligation of ubiquitin onto p27; however, the M and S analogues and TSA failed to inhibit the ubiquitination of p27 (Figure 1). This result suggests both the macrocycle and aliphatic chain are required for ubiquitin E1 inhibition. Furthermore, the result also suggests that the thioester moiety of Largazole is not required for inhibition, because the ketone and ester analogues were equally potent in blocking p27 ubiquitination. In addition, E1 inhibition is unrelated to HDAC inhibitor activity of Largazole as both ketone and ester failed to inhibit HDAC and TSA, a known HDAC inhibitor, does not block p27 ubiquitination *in vitro*. Prior to ubiquitination, p27 is phosphorylated by the Cdk2-CyclinE complex. We carried out an *in vitro* p27 phosphorylation assay in the presence of either DMSO or Largazole in order to test whether or not the decrease in p27 ubiquitination was due to the inhibition of the Cdk2-CyclinE complex. We observed that Largazole does not inhibit the phosphorylation of p27 (Figure 2b); therefore, the inhibition of p27-ubiquitin conjugation is due to an inhibition of the ubiquitination process rather than phosphorylation step.

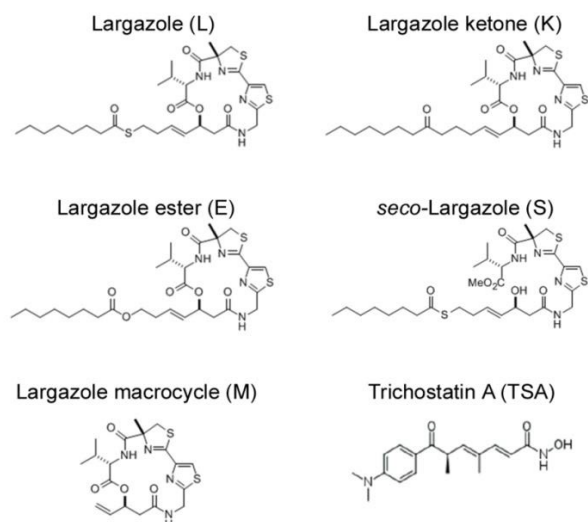


Figure 1. Chemical structures of Largazole, synthetic analogues, and Trichostatin A synthesized.

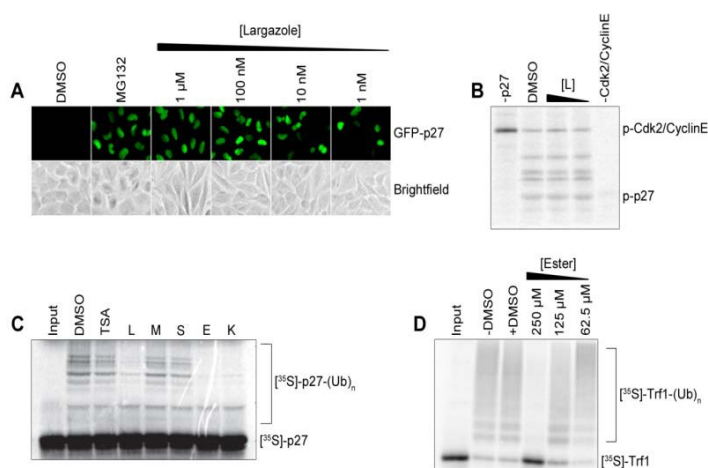


Figure 2. Largazole stabilizes p27 expression in Kip16 cells and inhibits p27 ubiquitination *in vitro* but not phosphorylation of Cdk2-CyclinE.

Even though there is a limited number of proteins in the reconstituted p27 ubiquitination system *in vitro*, tracing the real target of Largazole is still quite challenging. Fortunately, we have previously established another reconstituted *in vitro* ubiquitination assay of Trf1 with SCF^{Fbx4} (20). There are a few overlapping components between these two assays. The effect of Largazole on Trf1 ubiquitination should offer some insight as to where Largazole might target. To study the specificity of Largazole, we added Largazole ester to an *in vitro* Trf1 assay and found that Largazole ester inhibited the ligation of ubiquitin onto Trf1 in a dose-dependent fashion. Since Trf1 and p27 require different E2 ubiquitin-conjugating enzymes and different E3 ubiquitin-ligating recognition subunits in order to carry out each ubiquitination, we

hypothesized that Largazole and select synthetic analogues inhibit a step common to both ubiquitination pathways.

Largazole ketone inhibits ubiquitin E1 activation. In vertebrates, there exists only one known ubiquitin-activating E1 enzyme, UBA1. Since both p27 and Trf1 can be ubiquitinated in the presence of UBA1, we hypothesized that the inhibitory activity of Largazole is due to the deactivation of E1. To test this hypothesis, we incubated Largazole and Largazole ketone with recombinant E1 prior to carrying out an *in vitro* thioester assay we described previously (7). The presence of a fluorescence signal in the thioester assay suggests the formation of E1-ubiquitin adducts. The dose dependent decrease in fluorescence indicates that Largazole and Largazole inhibit the formation of E1-ubiquitin adducts (Fig 3AC). The dose-response curves generated from Figure 3BD suggest an IC_{50} of approximately 29 μ M and 25 μ M, respectively. Activated ubiquitin is normally transferred to ubiquitin conjugating enzymes (E2). If E1 activity is inhibited, we expect to see that defects in E1 activation should impair the attachment of ubiquitin onto Cdc34 (E2). To further validate E1 inhibition, we included Cdc34, the E2 enzyme required for p27 ubiquitination, in the E1 reaction mixture. As shown in Figure 3EF, in the presence of ATP, fluorescent ubiquitin is transferred to Cdc34 indicated by the presence of a fluorescent Cdc34 band on the gel. Upon incubation with E2, Largazole or Largazole ester reduce the amount of ubiquitin molecules that are transferred from E1 to E2 in a dose-dependent fashion. This result is consistent with the notion that Largazole or Largazole ester inhibit E1 activity.

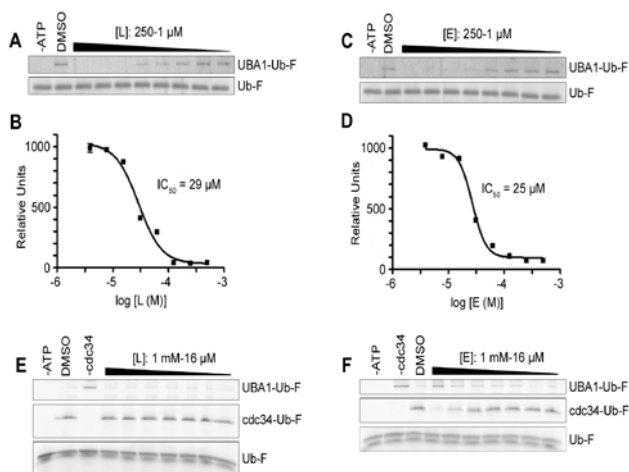


Figure 3. Largazole (L) and largazole ester (E) inhibit ubiquitin E1 in a dose dependent manner *in vitro*.

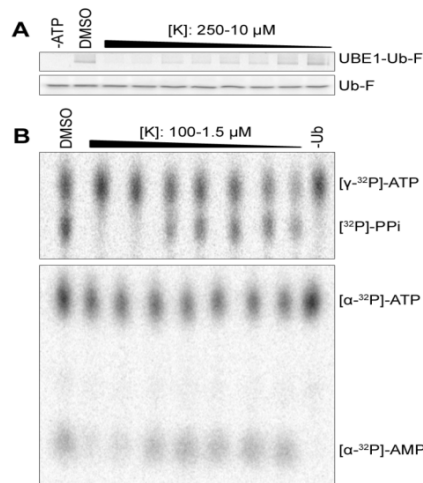


Figure 4. Largazole ketone inhibits the adenylation of the E1 ubiquitin-activating enzyme.

A potential caveat of the above experiment is that if Largazole or Largazole ester also blocks the transfer of ubiquitin from E1 to E2 we would have seen the same result. To rule out this possibility, we first produced ubiquitin charged E1 by incubating ATP and fluorescent ubiquitin for 30 min at room temperature followed by the addition of Cdc34, which was also contained with Largazole or Largazole ester. If either compound block ubiquitin transfer from E1 to E2, we would observe a significant decrease in Cdc34 fluorescence signal regardless of the order of compound addition. On the other hand, we should see the opposite results. As shown in Figure 3GH, Cdc34 is fully conjugated with fluorescence ubiquitin when Largazole or Largazole ester was added after generating fluorescent ubiquitin-E1. This result suggests that Largazole or Largazole ester neither blocks the transfer of activated ubiquitin from E1 to E2 nor promotes hydrolysis of ubiquitin thioester.

Largazole ketone inhibits the adenylation step of E1 activation. E1 forms an ubiquitin-adenylate intermediate during the course of its catalytic cycle (4). Thus the mechanism of ubiquitin E1 activation can be studied by assaying ATP:PPi and AMP:ATP exchanges (4). Production of AMP in the $[\alpha\text{-}^{32}\text{P}]\text{-AMP}:[\alpha\text{-}^{32}\text{P}]\text{-ATP}$ exchange assay guarantees that a thioester bond is formed between E1 and ubiquitin, while the release of PPi, measured by the $[\text{P}^{32}]\text{-PPi}:[\gamma\text{-}^{32}\text{P}]\text{-ATP}$ exchange assay, signals the formation of ubiquitin

adenylate. To further dissect the mechanism of Largazole inhibition, two nucleotide exchange assays were carried out in the presence of Largazole derivatives. For these experiments we used Largazole ketone, which is similar to Largazole and Largazole ester. From the results shown in Figure 4, it is evident that the first two concentrations of Largazole ketone (100 and 50 μM) inhibit ubiquitination of E1 similarly and were also inhibitory in both types of exchange assays. The lack of a [^{32}P]-PPi signal suggests that the adenylation step did not occur; consequently, ubiquitin could not be transferred to the active site cysteine to trigger the release of AMP. Both steps of the E1-catalyzed reactions can be measured by the AMP:ATP exchange assay. The lack of an [α - ^{32}P]-AMP signal further suggests that the adenylation step is inhibited by Largazole ketone. Thus Largazole or Largazole derivatives act on the first step of ubiquitin activation pathway by blocking the formation of ubiquitin-adenylate.

Selectivity of Largazole ketone against SUMO E1 and Uba1p. In

addition to ubiquitin, there exist several ubiquitin-like proteins that covalently modify other proteins. All of the ubiquitin-like proteins have activation pathways similar to ubiquitin (6). In order to study the specificity of Largazole to the ubiquitin pathway, we incubated Largazole ketone with SUMO-activating E1 enzyme prior to carrying out a thioester assay. From the results in Figure 5b, we found that Largazole ketone is ineffective in inhibiting the formation of E1-SUMO adducts. From the dose-response curve generated from the SUMO E1 fluorescence results, the IC_{50} is approximately 450 μM as opposed to 10 μM for ubiquitin E. Thus Largazole is relatively selective in perturbing ubiquitin E1 activation.

Ubiquitin and the ubiquitin E1 enzyme are highly conserved among eukaryotes (6). Sequence analysis shows a 45% homology between the human ubiquitin-activating enzyme E1 (UBA1) and *S. pombe* E1 (ptr3/Uba1p) at the amino acid sequence level. To test whether Largazole ketone inhibits the *S. pombe* E1, we carried out a thioester assay using Largazole ketone and the ubiquitin E1 homologue in *S. pombe*, Uba1p. The results in Figure 5a suggest that Largazole ketone fails to inhibit the formation of E1-ubiquitin adducts at concentrations less than 1 mM. Taken together, these results suggest that Largazole and its derivative are highly selective in inhibiting the ubiquitin E1 enzyme.

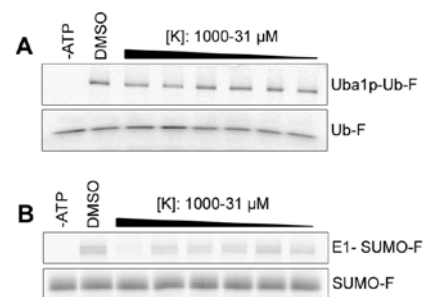


Figure 5. Investigation into the selectivity of Largazole ketone (K). A, Largazole ketone (K) fails to inhibit the ligation of ubiquitin onto Uba1p, a homologue of UBA1 from *S. pombe*. Formation of Uba1p-ubiquitin adducts was determined by thioester assay utilizing fluorescein-ubiquitin.

The results described above were published in *PloS ONE* in Journal earlier this year.

The foundations of these studies is our now highly developed and optimized synthesis route for the production of largazole, paragazole, largazole ketone, and other related compounds. In the past year we have attained capabilities that allow the production of gram amounts of compounds, and we have also developed a route to largazole and paragazole ketone analogs that allows for diversification at a late stage, facilitating the production of compounds that can be used to understand the features required for the inhibition of E1 (figure 6). We are also poised to commence the synthesis of folic acid and other targeted derivatives of largazole and congeners.

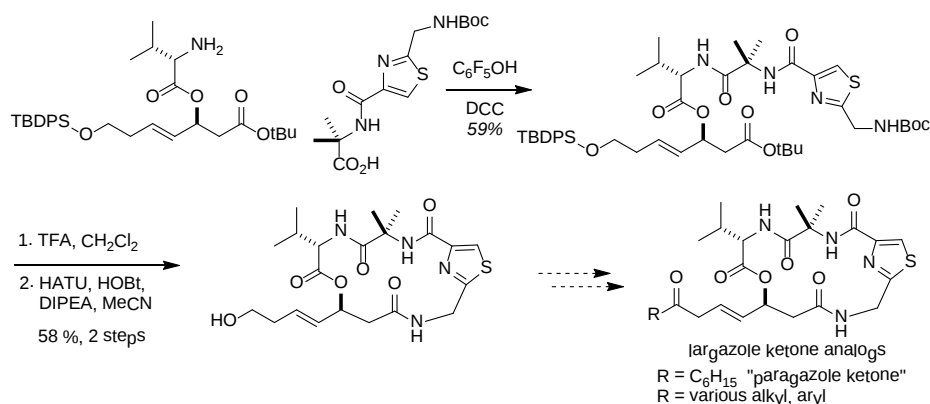


Figure 6. Optimized synthesis strategy for largazole ketone, paragazole ketone, and other analogs, exemplified by the route that is used to produce paragazole ketone. A variety of R groups can be appended.

Aim 2. To determine whether dual inhibition of both HDAC and ubiquitin conjugation is responsible for the selectivity of largazole against breast cancer cells and determine which HDAC isoforms render breast epithelial cells sensitive to largazole.

We have started the effort of cloning of all HDAC enzymes. So far we have cloned HDAC1, 2, 3, 4, 6, 8 and 11. We will continue to procure clones from ATCC to complete the HDAC enzyme set cloning project. The TGI assay on MDA-MB231 and a battery of breast cancer cell lines have been ongoing.

- Task 2.1 Cloning of 17 HDAC enzymes into lentiviral vector (**Liu, Months 1-4) (Completed).**
- Task 2.2 Construct human mammary epithelial cell lines (HME) expressing each individual HDAC enzyme (**Liu, Months 5-16) (Partially completed).**
- Task 2.3 Perform cell based growth inhibition assays using MDA-MB231 and HME cells using largazole analogs generated in Aim 1 (**Liu, Months 6-18) (ongoing).**
- Task 2.4 Measure cell permeability of largazole analogs using parallel artificial membrane permeability assay (**Liu, Months 6-18) (ongoing).**
- Task 2.5 Measuring the largazole sensitivity of HME cell lines expressing HDAC enzymes (**Liu, Months 12-18) (Completed).**
- Task 2.6 Perform siRNA and shRNA experiments for informative HDAC enzymes in HME and MDA-MB231 cells (**Liu, Months 19-24) (initiated).**
- Task 2.7 Write the manuscript describing dual targeting activity of largazole (**Liu and Phillips, Month 12, completed).**

We tested a panel of 18 breast cancer cell lines from a heterogeneous group of breast cancer cell lines. In our initial exploratory studies, we made an interesting observation that triple negative breast cancer (TNBC) cell lines are particularly sensitive to Paragazole inhibition. Since TNBC are associated with a shorter median time to relapse and death and significant unmet medical need due to the fact that these cancers do not respond to endocrine therapy or other available targeted agents, we decide to focus on testing TNBC cell lines instead of broader spectrum of breast cancer cell lines. We used a panel of 19 breast cancer cell lines to assess the proliferative response to increasing concentrations of Paragazole using an SRB assay. As shown in Figure 7, a majority of these cell lines are quite sensitive ($IC_{50} < 500$ nM) to Paragazole treatment although some are more sensitive than others. Several TNBC lines are inhibited at subnanomolar concentrations of Paragazole. These preliminary data suggests that Paragazole might be a novel agent to combat TNBC.

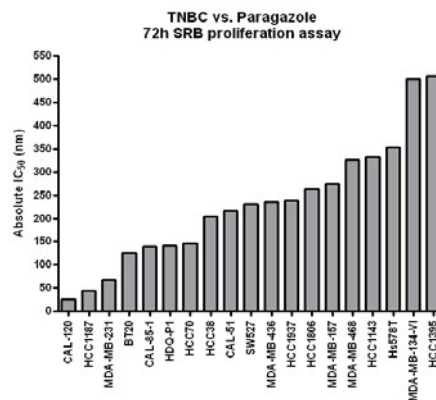
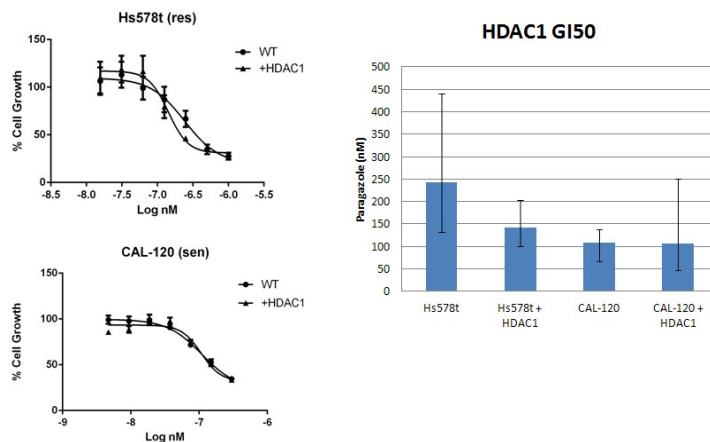


Figure 7. Growth Inhibition assays of Paragazole in TNBC lines.



Next we picked a sensitive cell line CAL-120 and a relatively growth resistance cell line Hs578t to stably over express HDAC1,3,6,8 in them using lentiviral mediate gene transfer. Of four enzymes, only overexpression of HDAC1 has observable differences in Largazole or Paragazole response. These differences are quite small and more rigorous studies are needed to establish that the effects are reproducible and statistically significant (Figure 8). Preliminary data does appear to support the hypothesis that overexpression of certain HDAC isoform may render cells more sensitivity to Largazole inhibition.

Figure 8. Growth Inhibition assays of Largazole sensitivity in cell lines overexpression of HDAC1.

Aim 3. To determine the chemotherapeutic efficacy of largazole to inhibit breast cancer growth and metastasis in mice

- Task 3.1 Large scale synthesis for largazole for initial testing along with control (Taxol) **(Phillips, Completed)**
- Task 3.2 Large scale synthesis of the improved lead for animal testing **(Phillips, Completed)**
- Task 3.3 Determining the chemotherapeutic effectiveness of largazole to prevent mammary tumor growth, invasion, and angiogenesis using the xenograft animal model **(Schiemann, ongoing)**
- Task 3.4 Assessing the effects of largazole to prevent mammary tumor metastasis **(Schiemann, ongoing)**
- Task 3.5 Testing newly improved largazole analogs in growth, invasion, angiogenesis and tumor metastasis in the xenograft animal model **(Schiemann, Months 12-23)**
- Task 3.6 Write and submit manuscripts for publication and final report to CDMRP **(Liu, Phillips and Schiemann, Month 24)**

Because Dr. William Schiemann, the collaborator on this project has moved his laboratory to Case Western Reserve University in Cleveland, which resulted in some delays getting underway (subcontract reworking, obtain approval for IACUC and animal protocols etc.). In addition, Professor Schiemann had to rebuild his research team at Case Western Reserve University since many experienced researchers in his lab did not follow him to Cleveland. Despite of these setbacks, Professor Schiemann's group has managed to undertake initial studies using paragazole (which shares similar antitumor activity to largazole) and is more readily prepared on scale. Using paragazole and GZ, a control kinase inhibitor, Professor Schiemann has shown 1) Paragazole potently inhibits the growth of human and murine TNBC organoids; 2) GZ potently inhibits the growth of human and murine TNBC organoids; 3) Preliminary evidence suggests that paragazole and GZ exhibit cytotoxicity against human MDA-MB-231 tumors in mice. A detailed discussion of these results can be found in Professor Liu's version of this yearly report. The preliminary studies clearly demonstrate significant promise in treating triple negative breast cancers *in vitro* and *in vivo*. Professor Schiemann is repeating these experiments and obtain enough data points to ensure the observations made are statistically significant. We expect to publish our results in the next 6-12 months.

3. KEY RESEARCH ACCOMPLISHMENTS

- We have completed synthesis initial round of largazole analogs for SAR testing.
- We have highly optimized chemistries for obtaining largazole, paragazole and analogs.
- We showed that Largazole and its analogs selectively inhibit ubiquitin E1 enzyme activity *in vitro*
- We demonstrated that inhibitory activity of Largazole is independent of its inhibitory activity towards the histone deacetylase enzymes
- Structure-activity relationship analysis shows that the thioester is not required for inhibition but the macrocycle core and aliphatic tail are required.
- Largazole blocks ubiquitin activation at the adenylation step and without perturbing ubiquitin transfer from E1 to E2.
- We show that Largazole inhibition of E1 is highly selective as it does not inhibit a highly related ubiquitin E1 enzyme from *S. pombe* and is almost twenty fold less effective in inhibiting the activation of SUMO E1.
- We showed that Largazole represents a new class of ubiquitin E1 inhibitor.
- We showed that Largazole and Paragazole potently inhibit the growth of human and murine triple negative breast cancer Organoids.
- Preliminary evidence suggests that Largazole and Paragazole exhibit cytotoxicity against Human MDA-MB-231 tumors in mice.
- Overexpression of single HDAC enzyme isoform is insufficient to convert sensitive cells to resistance or vice versa.

4. REPORTABLE OUTCOMES

Ungermannova D, Parker SJ, Nasveschuk CG, Wang W, Quade B, Zhang G, Kuchta RD, Phillips AJ, Liu X. Largazole and its derivatives selectively inhibit ubiquitin activating enzyme (e1). PLoS One. 2012;7(1):e29208.

5. CONCLUSIONS

We have made significant progress in our proposed studies. We demonstrated that Largazole is a new class of ubiquitin E1 inhibitor and the activity of E1 inhibition is independent of its inhibitory activity toward HDAC. It is possible to design novel dual inhibitors toward both pathways. We have published one manuscript and we expect to publish two additional manuscript on this project. We will continue to pursue the goals outlined in the original proposal in hope to develop a more effective anti-breast cancer drug candidate.

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