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PAPER

Synthetic Studies on the Indane SHIP1 Agonist AQX-1125

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AQX-1125 is an indane based SHIP1 agonist that has been evaluated in the clinic for the treatment of bladder pain syndrome/interstitial cystitis. To support our own studies on SHIP1 agonists as potential treatments for IBD and Crohn's disease, a new synthetic route to the SHIP1 agonist AQX-1125 has been developed. This sequence utilizes a hydroxy-acid intermediate which allows for ready differentiation of the C6 and C7 positions. The role of the C17 alkene in the biological activity of the system is also investigated, and this functional group is not required for SHIP1 agonist activity. While AQX-1125 shows SHIP1 agonist activity in enzyme assays, it does not show activity in cell based assays similar to other SHIP1 agonists, which limits the utility of this molecule.

Introduction

The phosphoinositide 3 kinase (PI3K) signaling axis is a major cell signaling pathway, trafficking information about the extracellular environment from outside the plasma membrane through the cytoplasm to the nucleus.[1] This signaling influences cell metabolism, effector functions, proliferation, and survival.[2] Membrane receptors mediate these effects, initiating signaling cascades through a network of enzymes and second messengers inside the cell, with phosphoinositides acting as participants.[3] The pattern of phosphorylation present on the phosphoinositide ring are detected by PH and C2 domains present in protein kinases and other signaling adapters leading to the transmission of information to the nucleus. Phosphatidylinositol-3,4,5-trisphosphate ($PI(3,4,5)P_3$ (Figure 1) is regarded as a key secondary messenger in the PI3K pathway. Normally $PI(3,4,5)P_3$ is maintained at a low concentration, but activation of PI3K leads to the local synthesis of $PI(3,4,5)P_3$ from $PI(4,5)P_2$, rapidly increasing the intracellular concentration of this phosphoinositide and activation of downstream signaling elements (e.g., Akt, mTOR).[4] Inositol phosphorylation is therefore closely regulated by inositol kinases and phosphatases.[5] Modulation of inositol kinases and phosphatases has become an active research area, as aberrant activation or loss of function in these enzymes is implicated in many disorders.[6] Inhibition of PI3K has been shown to have widespread influence on cellular physiology,[1] and several PI3K inhibitors are now being utilized as cancer treatments.[7] Resistance has already been documented,[6a, 8] however, leading to the investigation of alternative approaches to control signaling including the modulation of inositol phosphatases. The primary inositol phosphatases involved in processing $PI(3,4,5)P_3$ are PTEN (phosphatase and tensin homolog protein) and SHIP (src homology 2 (SH2) – containing inositol 5'-phosphatase).[9] Genetic studies have indicated that modulation of inositol phosphatase activity may influence the development and progression of disorders involving inflammation and cell division.[10] Although PTEN and SHIP both negatively regulate the PI3K pathway, they do so in different ways, with PTEN converting $PI(3,4,5)P_3$ to $PI(4,5)P_2$ while SHIP converts $PI(3,4,5)P_3$ to $PI(3,4)P_2$.[9a] By decreasing the cellular concentration of $PI(3,4,5)P_3$, activation of SHIP provides an alternative means to influence signaling.[11]

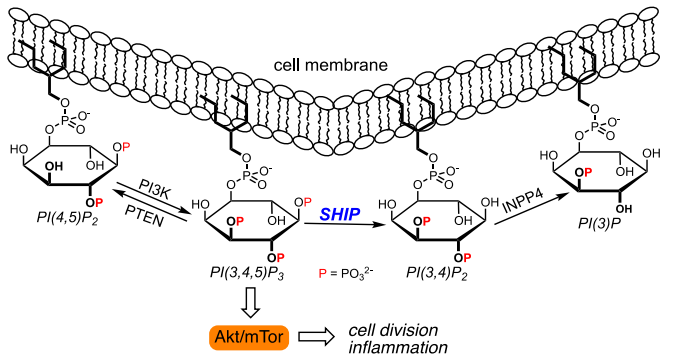

Figure 1 Modification of phosphatidylinositol second messengers by kinases and phosphatases in the PI3K pathway

The role SHIP plays in PI3K signaling has become an active research area.[12] One focus of this research has been the modulation of SHIP phosphatase activity with small molecules.[13] Both SHIP1 agonists (as antitumor[14] and anti-inflammatory agents[15]) and SHIP1 inhibitors (as antitumor agents,[16] immunotherapeutics,[17] and Alzheimer's

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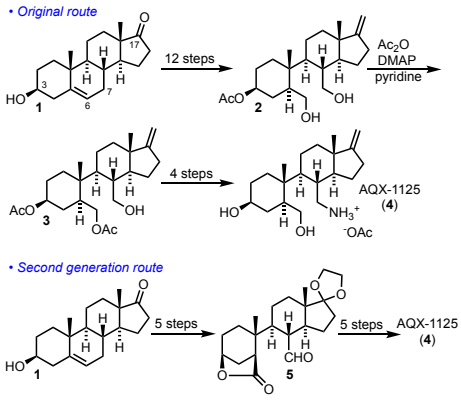
therapeutics[18]) have been investigated. The best known SHIP1 agonist is the indane AQX-1125 which was evaluated in clinical trials even though the molecule showed only moderate activation of the SHIP1 enzyme (~20% increase in SHIP1 activity at 300 μM in the Malachite Green assay[15]). AQX-1125 was evaluated in the clinic for the treatment of bladder pain syndrome/interstitial cystitis (BPS/IC),[19] but development was halted due to lack of efficacy. Given our recent results indicating that SHIP1 deficiency in the intestine correlates with severity in inflammatory bowel disease (IBD) and Crohn's disease,[20] we began a program to access known SHIP1 agonists to evaluate their effects in models of IBD. AQX-1125 was an obvious choice to use in our studies, as it is the only SHIP1 agonist to advance to the clinic, and therefore we undertook synthetic studies on the molecule to prepare AQX-1125 to evaluate its effects in IBD model systems.

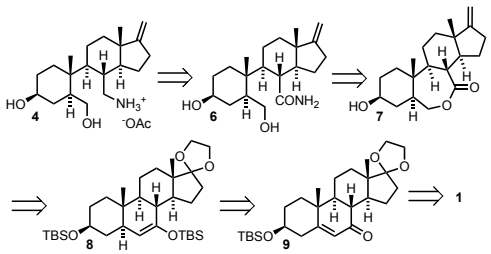
The first reported synthesis[21] of AQX-1125 begins with dehydroepiandrosterone **1** (Scheme 1). The route utilizes numerous functional group interconversions until intermediate **2** is reached. Diol **2** is then acylated selectively at the C6 hydroxyl(steroid numbering), as this alcohol is evidently less hindered. The acetate **3** is then taken on to the desired AQX-1125 **4**, with the entire route requiring 17 synthetic steps. Given the length of this route and the uncertainty about the selective acylation, we initiated our own synthesis of AQX-1125 to improve access to the molecule and prepare sufficient quantities for testing. After completion of our synthesis,[22] a number of different synthetic routes to AQX-1125 were disclosed in the patent literature.[23] The route presented with the most detail utilizes the lactone **5** as an intermediate, which differentiates C6 and C7 while also protecting the C3 alcohol. Our route is competitive in length with the second generation Aquinox route and proceeds through a different lactone intermediate. In this report we discuss our new route to AQX-1125, the testing results on new intermediates, and investigate the role of the C17 alkene on the biological activity.



Scheme 1 Reported Syntheses of AQX-1125

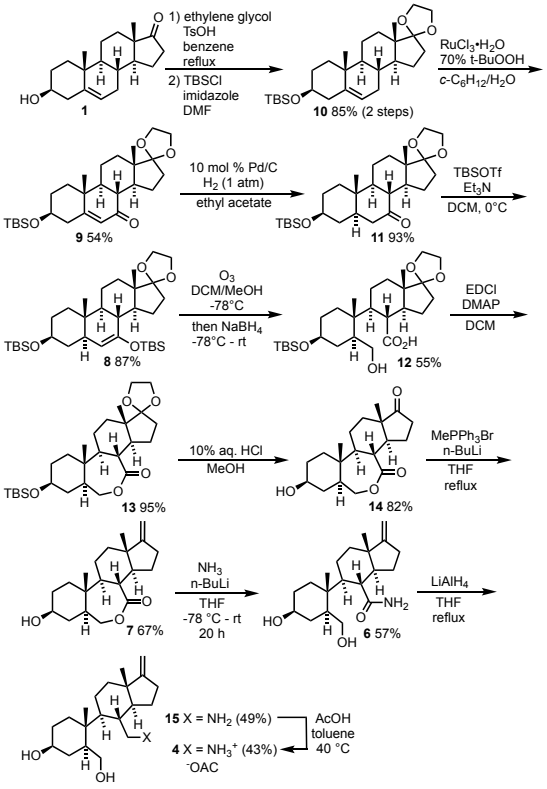
Results and Discussion

In order to evaluate AQX-1125 in our assays we developed a new synthetic route to the molecule. This new route utilized a different lactone intermediate to differentiate C6 and C7 positions. Beginning with dehydroepiandrosterone **1**, the synthesis would proceed through ketone **9**, which would be converted into the silyl enol ether **8** (Scheme 2). Oxidative cleavage of the silyl enol ether followed by cyclization would provide lactone **7**, which can provide access to amide **6** and eventually AQX-1125 **4**. This new route avoids the diol intermediate **2** and provides access to new analogs that can be evaluated for SHIP1 agonist activity.



Scheme 2 Retrosynthetic Analysis of AQX-1125

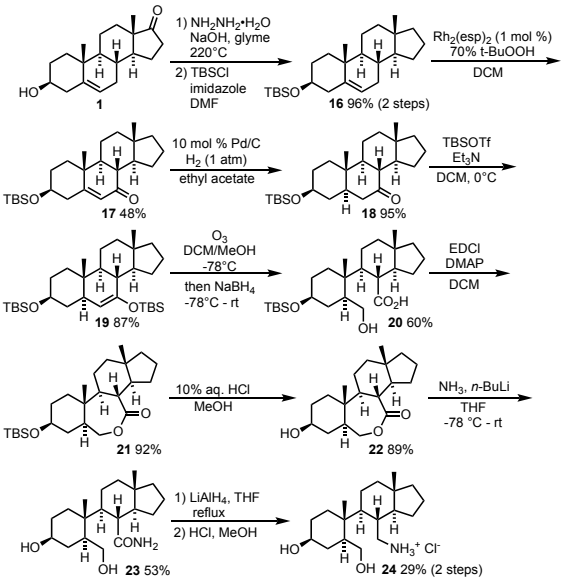
Following this plan, the C17 ketone of dehydroepiandrosterone **1** was protected as the ketal followed by formation of the silyl ether **10** (Scheme 3). Ruthenium-catalyzed allylic oxidation[24] of the C7 position then provided ketone **9**. Hydrogenation of the C5-6 alkene then gave ketone **11**. The stereochemistry of this transformation is well precedented,[25] leading to the addition of an α-hydrogen at C5. This outcome is rationalized by the catalyst avoiding the axial C10 methyl group. Formation of the silyl enol ether was then accomplished following a procedure from Deslongchamps[26] in 87% yield.



Scheme 3 New Synthetic Route to AQX-1125

Oxidative cleavage of the resulting silyl enol ether was then investigated, with ozonolysis being the most successful (two step protocols involving Rubottom oxidation and periodate cleavage gave lower yields). Direct reduction of the ozonide with sodium borohydride provided the hydroxy-acid **12** in 55% yield. The carboxylic acid **12** was then cyclized to the seven-membered lactone with EDCI. Simultaneous deprotection of the ketal and silyl ether was accomplished utilizing aqueous HCl in methanol leading to ketone **14**. A Wittig reaction then installed the C17 methylene providing alkene **7**. Opening of the lactone with lithium amide (formed *in situ* from anhydrous ammonia and *n*-butyllithium) provided the desired amide **6**, which could be reduced with $LiAlH_4$ to access amine **15**. Formation of the acetate salt then provided AQX-1125 (**4**).

In addition to providing material for our biological assays, the synthetic studies on AQX-1125 provided an opportunity to study some structure activity relationships. In particular, the role the C17 alkene played in the SHIP1 agonist activity was of interest, as this functional group was hypothesized to be uninvolved in interactions with the enzyme and lengthened the synthetic route. Should the C17 alkene not be required, the analog could also be utilized in our studies, providing a more expedient route to SHIP1 agonists. To explore this possibility, a synthesis of the analog **24** was undertaken (Scheme 4). The C17 ketone of dehydroepiandrosterone **1** was reduced to the alkane utilizing Wolff-Kishner conditions,[27] and the C3 alcohol protected as a TBS ether.



Scheme 4 Analog Synthesis

Oxidation of the C7 position utilizing Ru-catalyzed conditions resulted in an unexpectedly low 25% yield of enone **17** along with numerous side products. This was attributed to the greater solubility of enone **17**, as enone **9** precipitates from the reaction mixture whereas more soluble enone **17** stays in solution leading to overoxidation. Adopting the Rh-catalyzed conditions of Wang[28] gave a significantly higher yield of enone **17** (48%) with fewer side products. Hydrogenation of the alkene then provided ketone **18**. Formation of the silyl enol ether, oxidative cleavage and reduction of the ozonide with $NaBH_4$ led to the carboxylic acid **20**. Formation of the lactone with EDCI and removal of the silyl ether with HCl provided lactone **22**. Opening of the lactone with ammonia, reduction of the amide and formation of the HCl salt then provided analog **24**.

With AQX-1125 **4** and analog **24** in hand, their activity as SHIP1 agonists was evaluated using the Malachite Green assay[29] for phosphate release (Figure 2A). Both compounds function as SHIP1 agonists with similar potency, demonstrating a >50% increase in SHIP1 activity at 1 mM. This in vitro activity is consistent with the reported bioactivity of AQX-1125.[15a] The similar activity of these molecules indicates that the C17 alkene of AQX-1125 does not have a significant effect on SHIP1 activation. Evaluation of other intermediates on the route to AQX-1125 (**4**) and analog **24** (including **6**, **7**, **14**, **22** and **23**) showed that these molecules had no significant ability to accelerate the phosphatase activity of SHIP1, and also did not act as inhibitors of SHIP. The lack of activity of these systems as SHIP1 agonists indicates that a basic C7 amine is critical to agonist activity.

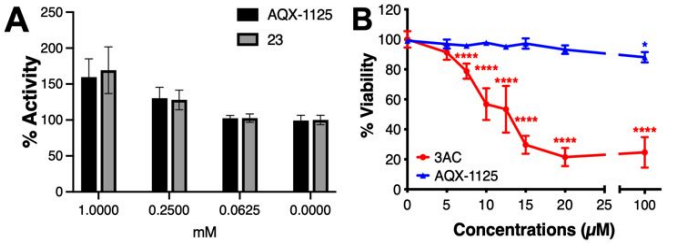


Figure 2 Bioactivity of AQX-1125 (**4**) and **24**. (**A**) SHIP1 agonist activity of AQX-1125 (**4**) and **23** in the Malachite Green Phosphatase Release Assay. (**B**) Effects of AQX-1125 (**4**) and 3α-aminocholestane on cell viability using OPM-2 multiple myeloma cells (DSMZ) as determined by Dojindo CCK-8 Cell Viability Assay. The significance of agonism for each compound vs. vehicle was assessed for all concentrations tested via a one-way ANOVA *p<0.05, ****p<0.0001.

AQX-1125 (**4**) was also evaluated for its effects on cell viability in the OPM-2 multiple myeloma cell line, which has been shown to express SHIP1.[30] AQX-1125 was reported to reduce phosphorylation of Akt in cells that express SHIP1 at concentrations as low as 10 μM,[15a] which often leads to a reduction in PI3K signaling and apoptosis. OPM-2 cells have been reported to undergo apoptosis when exposed to other classes of SHIP1 agonists[31] or SHIP1 antagonists,[16a] indicating a balance of both $PI(3,4,5)P_3$ and $PI(3,4)P_2$ is required for cancer cell survival.[32] Thus, OPM-2 cells are a useful model for evaluating the anti-tumor effects of SHIP modulators. AQX-1125 had little effect on cell viability at concentrations up to 100 μM (Figure 2B), however. In contrast, as reported previously, the SHIP1 antagonist 3α-aminocholestane (3AC) showed significant activity, inducing a 4-fold decrease in cell viability at 20 μM.[30] Both SHIP1 agonists and antagonists can induce cell death by induction of cell-intrinsic[30] or -extrinsic apoptosis,[20b] but the lack of activity of AQX-1125 is difficult to rationalize. Recently Mui and co-workers demonstrated that AQX-1125 is ineffective in treating inflammation in IL-10 knockout mice, while more potent SHIP1 agonists are effective

in this model.[33] This report also showed that AQX-1125 only binds to SHIP1 weakly, with SHIP1 perhaps not being the primary cellular target of the molecule. These results may explain the lack of activity of AQX-1125 on OPM-2 cells.

Conclusion

In summary we have developed a new synthetic route to the AQX-1125 that proceeds through a lactone intermediate to differentiate the functionality at C6 and C7. We also present in vitro testing results on the intermediates from the synthesis, and determined that the C17 alkene is not required for bioactivity. A simplified analog without the C17 alkene showed similar bioactivity. Preliminary evaluation of AQX-1125 showed that while the molecule appears to be a SHIP1 agonist in enzyme assays, it does not display cytotoxic effects against OPM-2 cells as was reported for other SHIP1 agonists. This is similar to the lack of activity in anti-inflammatory assays that was recently reported by Mui and co-workers.[33] The poor results in cell based assays indicate a need for more potent SHIP1 agonists with better performance in cell based assays and in vivo. We have therefore turned our attention to alternative SHIP1 agonist scaffolds for evaluation in cancer, IBD, and colitis models, as the AQX-1125 system appears to have little utility in these areas.

Experimental

An experimental section, including detailed procedures, ^{1}H and ^{13}C NMR spectra, is provided as ESI.

Conflicts of interest

S.D., S. F-D., W.G.K. and J.D.C. have patents on small molecules targeting of SHIP1 and SHIP2 in disease. The other authors have no conflicts to disclose.

Acknowledgements

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