Synthesis of Phosphatidic Acid Mimetics for Competitive Binding with the Receptor AFAP-110 in a Pathway Relevant to Breast and Ovarian Cancer

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Background

Activation of the tyrosine kinase cSrc is observed in increased amounts in cases of breast and ovarian carcenoma. When complexed to AFAP-110, cSrc is inactivated via an autoinhibitory conformation. The kinase is normally activated during the G2/M transition in the cell cycle, although it can be independently activated by other agents that release autoinhibition. PKCα, phosphatidic acid and phosphoinositides can cooperatively interact with the PH domain of AFAP-110 to affect the activation of cSrc.

1. AFAP-110

Largely responsible for stress filament cross-linking, AFAP-110 has a proline-rich SH3 domain, suiting it as a possible cSrc binding partner.

2. cSrc

cSrc is a tyrosine kinase typically found in the perinuclear vesicles of cells. Ativation of cSrc is known to promote the formation of invasive structures.

3. Autoinhibition

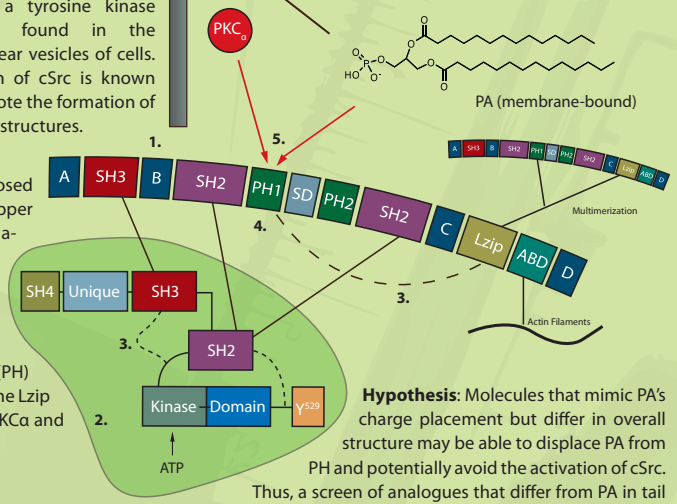
When inactive, AFAP assumes a closed conformation in which its Leucine-zipper (Lzip) moiety contacts PH. This conformation keeps cSrc inactive by allowing SH3 to contact cSrc's linker sequence, concealing the kinase domain.

4. PH Domains

AFAP-110 has two plekstrin homology (PH) domains. PH1 is allosterically linked to the Lzip moiety until AFAP-110 is activated by PKCα and releases this binding.

5. cSrc Activation

PKCα can phosphorylate AFAP on the PH domain, opening the conformation of AFAP. AFAP then travels to the membrane where phosphatidic acid (PA) and phosphoinositides (Ptdins) bind to PH. This associates AFAP with the membrane and allows for AFAP to approximate cSrc and activate it.



Goal

Phosphatidic Acid (PA)

PA is a membrane-bound molecule with a highly electron rich phosphate head group and a pair of long carbon chain tails. The specificity of binding to the pleckstrin homology (PH) binding pocket of AFAP-110 has been found to have a Kd of 12.5 μM.

The moment of interest in the cSrc activation mechanism is after PKCα-activation of AFAP, but before membrane-bound PH can bind to AFAP where it approximates and activates cSrc.

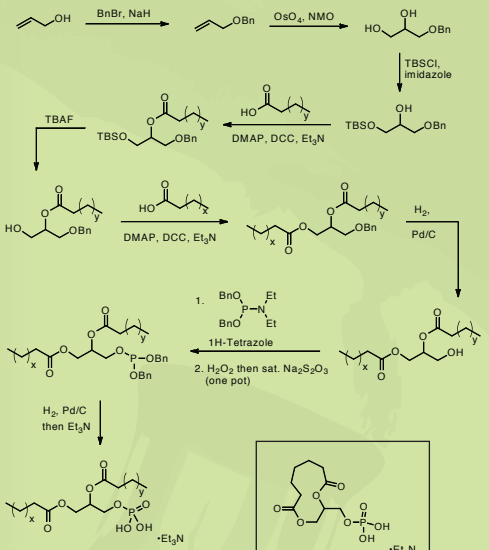
PA (membrane-bound)

Hypothesis: Molecules that mimic PA's charge placement but differ in overall structure may be able to displace PA from PH and potentially avoid the activation of cSrc. Thus, a screen of analogues that differ from PA in tail structure should be examined

What we're doing: The analogues will be synthesized and then passed through a Biacore surface plasmon resonance device to determine binding strength.

Synthesis

1. PA analogues



x	y
2	4
2	6
4	2
4	4
4	8
6	6
8	8

Progress: Thus far, 7 stable phosphates have been synthesized and sent to our collaborators to determine the binding strength to AFAP.

Macrolactone analogues are also being synthesized in hopes that their cyclic structure can increase control and binding specificity.