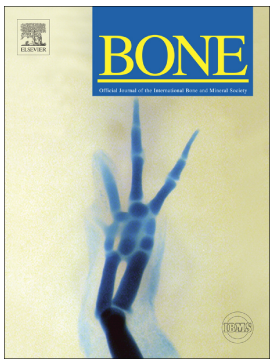

Hypoxia-selective allosteric destabilization of activin receptor-like kinases: A potential therapeutic avenue for prophylaxis of heterotopic ossification

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Hypoxia-selective allosteric destabilization of activin receptor-like kinases: A potential therapeutic avenue for prophylaxis of heterotopic ossification

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Highlights

- Activin receptor-like kinases regulate through intrinsic conformational plasticity.
- Small molecule modulators were screened by docking at the allosteric αC-β4 loop.
- In orthogonal *in vitro* assays, related compounds destabilized and inhibited ALK2.
- BMPRII kinase was resistant to H-SAAD/Ds, and in complex, protected the ALK.
- Destabilizers were more efficacious at hypoxic *vs.* normoxic pH, hence selective.

Abstract

Heterotopic ossification (HO), the pathological extraskeletal formation of bone, can arise from blast injuries, severe burns, orthopedic procedures and gain-of-function mutations in a component of the bone morphogenetic protein (BMP) signaling pathway, the *ACVR1*/ALK2 receptor serine-threonine (protein) kinase, causative of Fibrodysplasia Ossificans Progressiva (FOP). All three ALKs (-2, -3, -6) that play roles in bone morphogenesis contribute to trauma-induced HO, hence are well-validated pharmacological targets. That said, development of inhibitors, typically competitors of ATP binding, is inherently difficult due to the conserved nature of the active site of the 500+ human protein kinases. Since these enzymes are regulated via inherent plasticity, pharmacological chaperone-like drugs binding to another (allosteric) site could hypothetically modulate kinase conformation and activity. To test for such a mechanism, a surface pocket of ALK2 kinase formed largely by a key allosteric substructure was targeted by supercomputer docking of drug-like compounds from a virtual library. Subsequently, the effects of docked hits were further screened *in vitro* with purified recombinant kinase protein. A family of compounds with terminal hydrogen-bonding acceptor groups was identified that significantly destabilized the protein, abolishing activity. Destabilization was pH-dependent, putatively mediated by ionization of a histidine within the allosteric substructure with decreasing pH. *In vivo*, nonnative proteins are degraded by proteolysis in the proteasome complex, or cellular trashcan, allowing for the emergence of therapeutics that inhibit through degradation of over-active proteins implicated in the pathology of diseases and disorders. Because HO is triggered by soft-tissue trauma and ensuing hypoxia, dependency of ALK destabilization on hypoxic pH imparts selective efficacy on the allosteric inhibitors, providing potential for safe prophylactic use.

Keywords: bone morphogenetic protein, BMP, protein kinase, allosteric, R-spine, αC-β4 loop, *ACVR1*, ALK2, FKBP12, BMPRII, heterotopic ossification, fibrodysplasia ossificans progressiva, FOP, hypoxia, hypoxic pH, pharmacological chaperone, selective degrader, hydrophobic tagging, PROTACs, macrocyclic inhibitor, covalent inhibitor, kinase inhibitor.

Abbreviations: ALKs, activin receptor-like (type I) kinases; BMP, bone morphogenetic protein; BMPRII, BMP type II receptor; FKBP12, FK506-binding protein 12 kDa; FOP, Fibrodysplasia Ossificans Progressiva; AMPK, AMP-activated protein kinase; SMKI, small molecule kinase inhibitor; H-SAAD/D, Hypoxia-Selective ALK Allosteric Destabilizer/Degrader; SERDs, Selective Estrogen Receptor Degraders; PROTACs, PROteolysis-TArgeting Chimeras.