

SOUTHERN RESEARCH

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Abstract No. 4021

Novel small molecules targeting endothelial differentiation

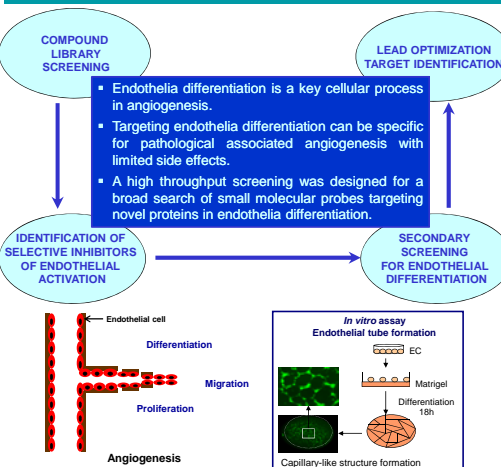
Anshu M. Roy, Ling Zhai, E. Lucile White, Subramaniam Ananthan, Rongbao Li and Zhican Qu

Southern Research Institute, Birmingham, Alabama

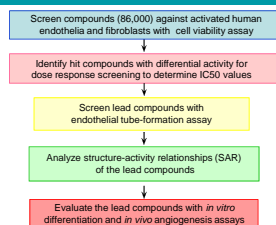
Abstract

Endothelial differentiation is a key step in the angiogenesis process, which is crucial for new blood vessel formation. However, molecular mechanisms through which endothelial cells differentiate to form elongated tubules leading to vessels allowing blood flow are not well understood. Endothelial cells are generally inactive during adulthood and abnormally active in tumors and other diseases and therefore agents that target endothelial differentiation can be specific for the pathological conditions with minimal side effects. To discover molecular probes of endothelial differentiation signaling pathways and to develop novel anti-angiogenic agents, we developed a system to identify small molecules that selectively inhibit endothelial differentiation, and thereby block excessive angiogenesis. A high throughput screening cell viability assay was developed and used for screening a large compound library against both human endothelia and fibroblasts. A group of hit compounds was selected based on their differential activity against endothelia versus fibroblasts and further screened for their potency in endothelial tube-formation assay. A number of compounds were identified as potent inhibitors of endothelial differentiation and moderate inhibitors of endothelial proliferation but had much less effect on fibroblasts. These compounds are currently being further studied with *in vivo* angiogenesis assays. Small molecular probes that selectively interact with biological targets are valuable research tools for understanding functions of proteins and other biomolecules and for elucidating biological pathways. Identification of protein targets of the selected compounds and study of mechanism of drug action are in progress.

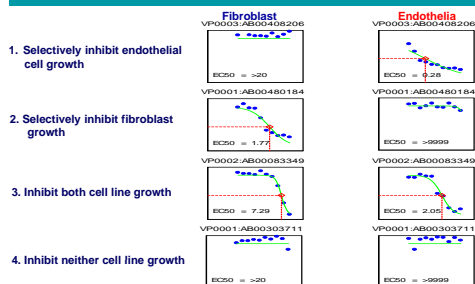
Introduction



HTS Flow Chart



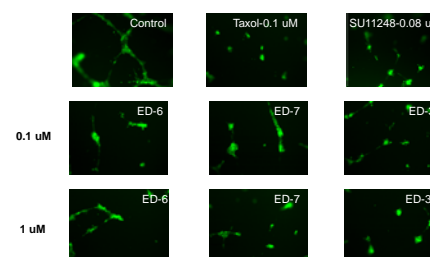
Categories of Selected HTS Hit Compounds



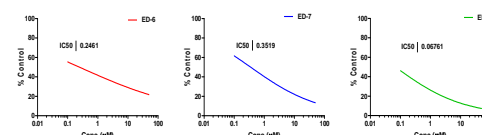
Activity of Selected HTS Lead Compounds

Angio ID	Object ID	Structure	<i>In vitro</i> Proliferation Assay (IC50- μ M)			
			endothelia (HUVEC)	lung fibroblast (LL47)	prostate epithelial (PWR-1E)	lung epithelial (BEAS-2B)
ED-6	AB00573290		3.5	20	36.1	>50
ED-7	AB00573350		3.4	22	42.5	>50
ED-35	AB00402053		6.7	>50	36.4	38.9

Activity of Lead Compounds in Endothelial Cell Differentiation



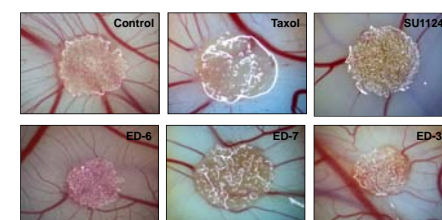
IC50 graphs for activity of Representative Hit Compounds in HUVEC Differentiation



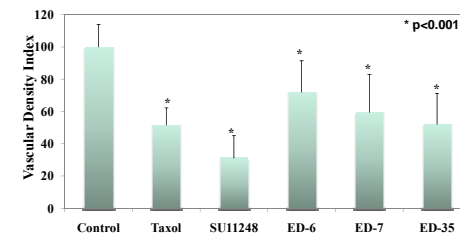
Summary of *In vitro* Activity of Lead Compounds in Endothelial Cells

Angio ID	IC50- μ M		
	Proliferation	Tube Formation	Migration
ED-6	3.5	0.25	17.94
ED-7	3.4	0.35	6.37
ED-35	6.7	0.07	4.40
SU11248	1.2	0.11	15.4

Activity of Lead Compounds in *In Vivo* Xenograft Tumor CAM Assay



Xenograft Tumor CAM assay: 1×10^6 MCF-7 cells were mixed with/without 2.5 μ g of each compound/CAM in Matrigel™ and added topically on the chick chorio-allantoic membrane (CAM) on Day 6, and vascular density index (number of intersections at the periphery of the tumor with blood vessels) was computed after 72 h.



Summary and Conclusions

- Several small molecules with sub micromolar IC50 values for endothelial differentiation have been identified.
- The lead compounds have shown inhibition of new blood vessel formation in xenograft-CAM assay.
- Identification of the protein targets is in progress, which could lead to a better understanding of endothelial cell differentiation.