

## Screening for Specific Anti-angiogenic Agents

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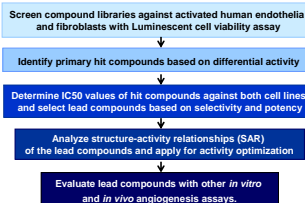
### Abstract

Imbalance in angiogenesis is involved in many pathological conditions, such as cancer, rheumatoid arthritis, and inflammation. Targeting angiogenesis has recently emerged as a proven therapeutic strategy for treatment of cancer and age-related macular degeneration. To understand the underlying biological signaling pathways of angiogenesis and to develop potential anti-angiogenic therapy, we have developed a system to discover small molecular probes that can selectively inhibit endothelial cell activation, and thereby block excessive angiogenesis. **First**, a high throughput screening (HTS) assay was developed for screening large libraries of chemical compounds against both primary human endothelia and fibroblasts to identify compounds with differential inhibitory activity against the endothelial cells versus fibroblasts. **Second**, HTS dose response-based EC50 values were determined to identify lead compounds with selectivity and potency against human endothelial activation. **Third**, a number of selected lead compounds were studied for their activities in endothelial cell tube-formation and migration, and to elucidate their mechanisms of action. **Fourth**, the selective compounds are being further studied *in vivo* by embryo CAM assay and mouse Matrigel plug assay. A number of compounds that exhibited specific inhibitory activities against human endothelial cells were identified from an 86,000 compound library screening. Analysis of structural clusters of the initial hits led to the identification of several chemical scaffolds as interesting leads. Structure-activity relationship (SAR) analyses and synthetic efforts on selected scaffolds for lead optimization are in progress. Our progress toward identification of specific anti-angiogenic agents will be presented.





### Introduction

To discover new molecular probes for the study of endothelial cell activation and potential new therapeutic development, Southern Research Angiogenesis Program developed a two-cell line based assay for High Throughput Screening of compound library to identify lead compounds with differential inhibitory activity against human endothelial cells versus human fibroblasts. Chemical libraries of 10,000 compounds and 86,000 compounds have been screened successfully with the developed assay at Southern Research HTS Center. Number of lead compounds with selectivity and potency against human endothelial activation have been identified, which are currently being further investigated *in vitro* and *in vivo*.

### Screening Flow Chart

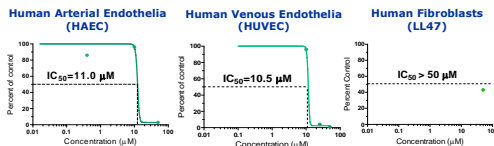


### Selective HTS Hit Compounds

#	Compound Name Molecular Wt	Structure	Inhibition HUVEC proliferation HTS 10uM	Inhibition LL47 proliferation HTS 10uM
1	Bithionol MW 356.1		98.7%	32.9%
3	Ritanserine MW 477.6		98.3%	12.6%
4	Amiodarone hydrochloride MW 681.78		97.2%	37.3%
6	Terfenadine MW 471.7		97.3%	98.1%

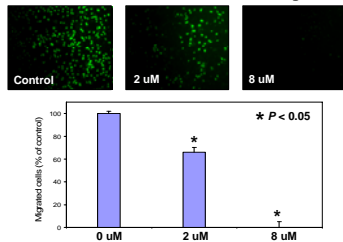
### Cell Proliferation Assay

#### Ritanserine Selectively Inhibits Endothelial Cell Proliferation



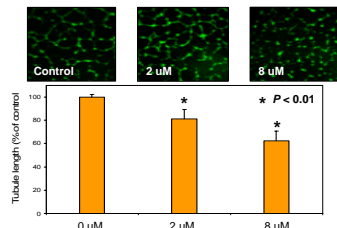
### Endothelial Cell Migration Assay

#### Terfenadine Inhibits Endothelial Cell Migration



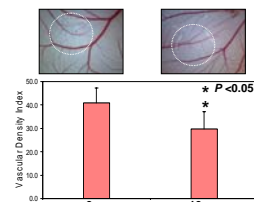
### Endothelial Tube Formation Assay

#### Bithionol Inhibits Endothelial Cell Tube Formation

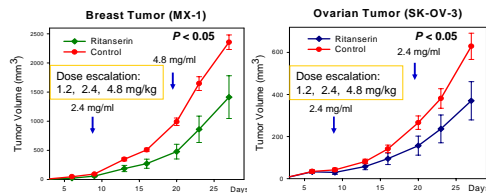


### Chick Chorio-Allantoic Membrane Assay

#### Amiodarone hydrochloride Inhibits Angiogenesis

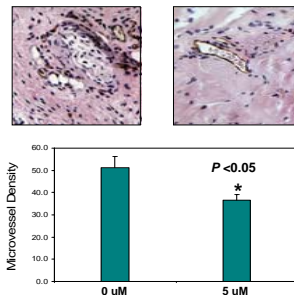


### Ritanserine Inhibits Xenograft Tumor Growth



### Matrigel Plug Assay

#### Ritanserine Inhibits Angiogenesis



### Anti-Angiogenic activities

Compound	IC <sub>50</sub> (μM) arterial endothelia proliferation	IC <sub>50</sub> (μM) venous endothelia proliferation	IC <sub>50</sub> (μM) Fibroblasts proliferation	Inhibition in Endothelia migration		Inhibition in Endothelia Tube-formation		Inhibition in CAM assay (10ug)
				2uM	8uM	2uM	8uM	
Ritanserine	11.0	10.5	>50	55%	65%	11%	33%	21%
Amiodarone hydrochloride	7.5	8.8	20.0	36%	38%	33%	33%	27%
Terfenadine	3.0	5.5	7.0	34%	100%	20%	27%	28%
Bithionol	2.0	6.0	10.5	5%	100%	19%	38%	30%

### Conclusions

Chemical libraries of 10k and 86k compounds have been screened using an anti-angiogenic cell-based viability assay. Number of HTS hit compounds have been identified and subjected to further *in vitro* and *in vivo* studies. Chemical synthesis for structural optimization of lead compounds has been initiated.