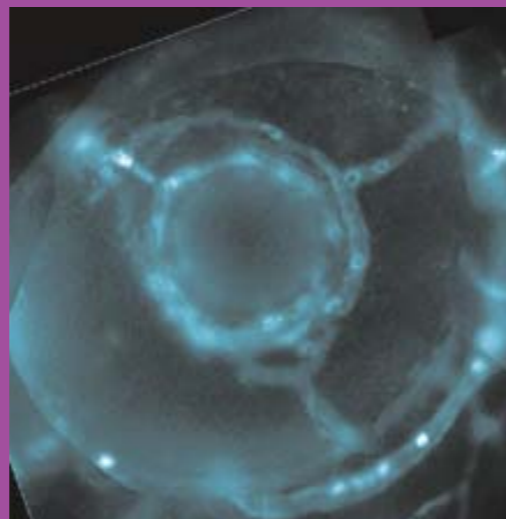




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Eye vessels stained by  
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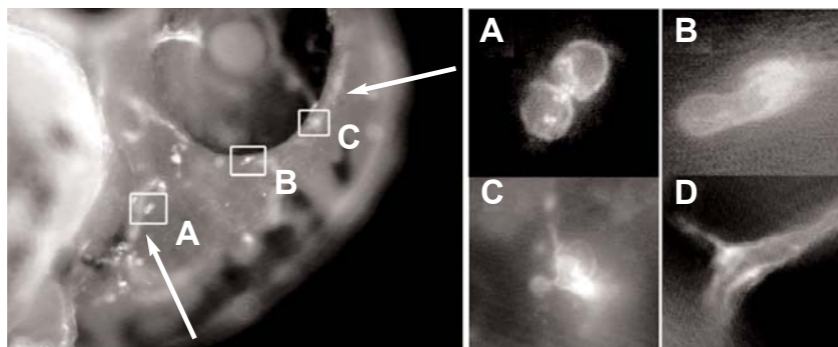
## ZEBRAFISH ANGIOGENESIS BIOASSAYS



Angiogenic vessel staining using mAb specific for activated endothelial cells.

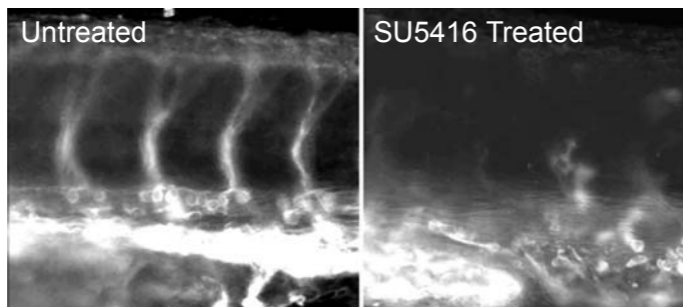


### ACTIVATED ENDOTHELIAL CELLS



Zebrafish were stained with activated endothelial cell (EC) specific mAb. Left panel shows several vessels forming in the head region (long white arrows). A, B, C show the white squares in the left panel at higher magnification. D is an image of a single intersegmental vessel (ISV) in the trunk. At higher magnification, individual cells are identifiable. Staining is strongest in the dividing and budding regions of the cells (A,B,C,D).

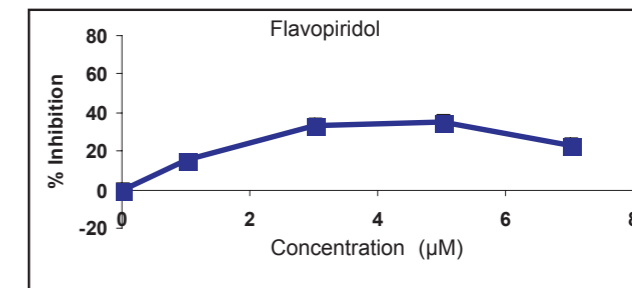
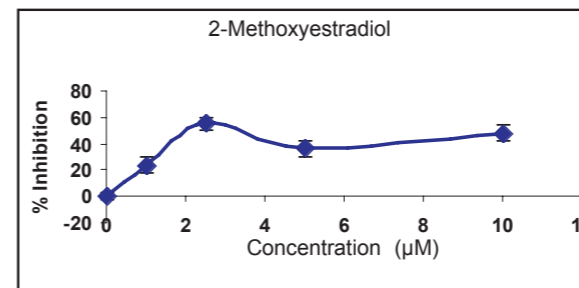
### VESSEL INHIBITION



After treatment with SU5416, tail ISVs were almost completely inhibited.

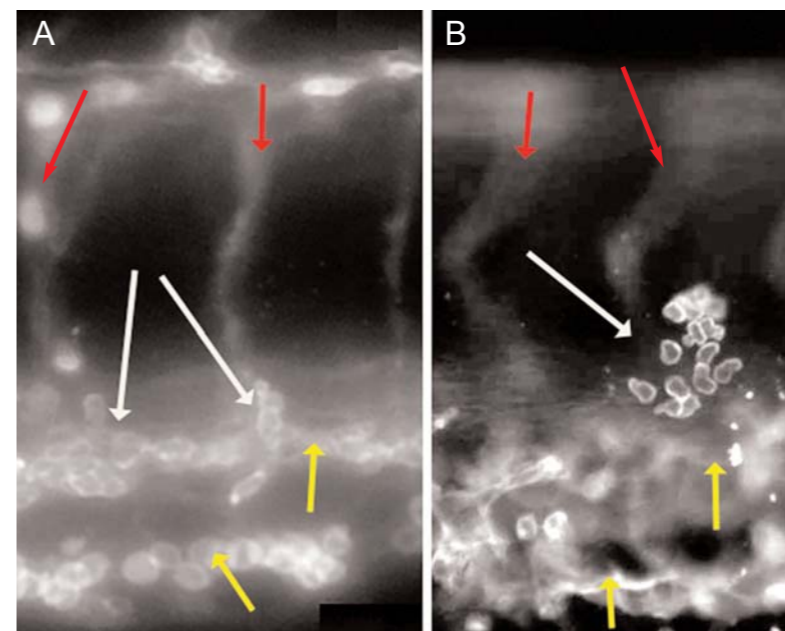
Methods of screening agents for activity using teleosts are covered by US patents: 6,299,858 and 6,656,449 owned by Phylonix.

### QUANTITATIVE WHOLE ANIMAL MICROPLATE FORMAT



2-Methoxyestradiol (2-ME) and Flavopiridol were used to validate the quantitative zebrafish *in vivo* ELISA EC proliferation assay. Percent inhibition of EC proliferation increased proportionately with drug concentration. Zebrafish were treated for 28 hours, one embryo per microwell.

### ZEBRAFISH *IN VIVO* EC MIGRATION ASSAY



Zebrafish were untreated (Control, A) or treated with 600 µM Tetracycline (B), then fixed and processed for whole mount mAb staining. In control embryo (A), mAb stained cells (white arrows) are aligned at the intersection of the normal caudal vessel plexus (CVP) (yellow arrows) and ISVs (red arrows). After drug treatment (B), ISVs are inhibited (red arrows), cells are shown aggregating (white arrow), and the CVP is abnormal (yellow arrows).