

Structure-activity relationship of a novel family of cysteinyl leukotriene receptor antagonist quinoline compounds with anti-angiogenic activity.

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Introduction: Previously, we identified quininib (2-[(*E*)-2-(quinolin-2-yl)vinyl]phenol), a cysteinyl leukotriene receptor antagonist with anti-angiogenic and anti-permeable activity (1,2). Here, we report a structure activity relationship study to more comprehensively characterise features which confer anti-angiogenic activity.

Methods: 70 quininib analogues were synthesised or purchased (3). Anti-angiogenic activity was ranked based on ability of 10 μ M compound to inhibit blood vessel formation in two *in vivo* developmental angiogenesis assays in Tg[*flil*:EGFP] zebrafish (i) hyaloid/ocular vessel formation and (ii) intersegmental /tail vessel formation. Compounds were considered active where \geq 40% inhibition was observed compared to control. Selected highly ranked compounds were tested in human endothelial cell lines (HMEC-1) for cytotoxicity (MTT assay) and anti-angiogenic activity (*in vitro* tubule formation assay). Efficacy was also determined in mouse aortic ring assay (4). CYSLT receptor antagonism was quantified using reporter cell bioassays.

Results: 18 analogues showed \geq 40% inhibition in formation of hyaloid vessels and fourteen showed \geq 40% inhibition in formation of intersegmental vessels. These compounds comprised those with "triene" bond and benzene ring modifications. Six compounds were selected for further testing (Q8: (*E*)-2-(2-Quinolin-2-yl-vinyl)benzene-1,4-diol HCl; Q 14: 3-(2-Quinolin-2-yl-ethyl)-phenol HCl; Q 18: 2-[(*Z*)-2-(Quinolin-2-yl)vinyl]phenol HCl; Q 20: (*E*)-2-(2-Quinolin-2-yl-propenyl)-phenol HCl; Q 22: 2-Quinolin-2-yl-ylethynyl-phenol HCl and Q 54: 2-(2-(2-Methoxyphenyl)vinyl)quinoline). No compound showed an effect on cell viability. Three compounds (Q 8, Q 18, Q 22) showed \geq 40% reduction in *in vitro* tubule length and Q 18 showed \geq 40% reduction in sprout formation in the aortic ring assay. Cell bioassays showed all six compounds inhibited LTD₄ induced activation of CYSLT₁ (IC₅₀: 1.7 - 6.7 μ M) and at 30 μ M, four compounds (Q 14, Q 18, Q 20, Q 22) showed \geq 50% inhibition of LTC₄ induced activation of CYSLT₂.

Conclusions: More than 10 compounds showed robust anti-angiogenic activity in both assays. Three quinoline compounds demonstrated anti-angiogenic activity in human cell lines and mouse aortic ring. This study is the first step in identifying a quinoline pharmacophore for antagonism of the CYSLT pathway as a therapeutic target for inhibiting the aberrant pathological angiogenesis occurring in blindness, cancer and arthritis.

References

1. Galvin O *et al.*, (2016). *J Control Release* **233**, 198-207.
2. Reynolds AL *et al.*, (2016). *J Biol Chem* **291**, 7242-7255.
3. Kennedy BN *et al.*, (2016) *USPTO* US 9,388,138 B2 (Patent).
4. Baker M *et al.*, (2012) *Nat. Protoc* **7**, 89-104.