# Novel compounds targeting PFKFB3, the key glycolytic enzyme, as a way to inhibit angiogenesis

**Stefano Bellosta[a,d], Alberto Corsini[a,d], Carlo de Dominicis[b], Matteo Zanda[b], Maria Luisa Gelmi[c], Anahita Abdali[a]**

[a]Dept. of Pharmacological and Biomolecular Sciences, Milan, University of Milan
[b]Institute of Medical Sciences, Aberdeen, University of Aberdeen
[c]Dept. of Pharmaceutical Sciences, Milan, University of Milan
[d]IRCCS MultiMedica, Milan, Italy

stefano.bellosta@unimi.it

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Angiogenesis is an important contributor to atherosclerotic plaque growth and instability. Clinical evidence has linked intraplaque angiogenesis with progressive and unstable vascular disease. Proliferating endothelial cells (ECs) can switch their metabolism to being highly glycolytic enabling their growth and division in the angiogenic process. Recent studies have demonstrated the therapeutic potential of 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), a commercially available 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB3) inhibitor, in angiogenesis models. For this reason, PFKFB3 inhibitors seem promising compounds to be used in promoting plaque stability.

In the present study, we studied the *in vitro* effects of the PFKFB3 inhibitor 3PO, and of two self-synthesized inhibitors named phenoxindazole analogues (PA-1 and PA-2; based on *Boyd et al., 2015*) on glycolysis, cell proliferation, migration, matrix metalloproteinase (MMP) activity and gene expression in ECs. We observed that these compounds were able to significantly reduce glycolysis levels in the human endothelial cell line EA.hy926. In addition, all three compounds markedly reduced endothelial cell migration, proliferation and wound closing capacity which are essential for neovessel formation. Moreover**,** we demonstrated by gelation gel zymography that these inhibitors reduce the activity of proMMP-9 and MMP-2 up to 40-50% and 20-30% compared to control, respectively. Furthermore, real-time PCR results indicate that the PA compounds downregulate PFKFB3 gene expression whilst 3PO does not. As for markers of migration and angiogenesis, such as ICAM and VEGFR2, these

were markedly reduced. Finally, gelatinase gene expression was downregulated by up to 80%.

These findings show that PFKFB3 inhibition with PA compounds markedly reduce endothelial cell migration, proliferation and gelatinolytic activity concomitant with a significant decrease in gelatinase gene expression, EC migration and angiogenesis markers. Thus, these compounds have the potential to be tested in an animal model of angiogenesis. This project has been funded by the European Union's Horizon 2020 Marie Skłodowska-Curie grant (#67552).