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# Development and application of a cell-based assay to allow the study of selective inhibitors of Hypoxia Inducible Factor (HIF) Prolyl Hydroxylase 1 (PHD1)

**Introduction:** Hypoxia Inducible Factor (HIF) is a heterodimeric transcription factor that drives the expression of genes involved in the hypoxic response. It is negatively regulated by a family of 2-oxoglutarate-, Iron- and Oxygen- dependent Prolyl Hydroxylases (PHD1, PHD2 and PHD3). Inhibition of PHDs by a non-selective inhibitor has a potential utility in the treatment of ischemic disease but has the unwanted side effect of increasing haematocrit through the stimulation of EPO expression. Clinical studies and mouse knock-out work suggests that a selective inhibitor of PHD1 may be a point of pharmacological intervention in both ischemic diseases (1,2) and also in Irritable Bowel Disease (IBD)(3,4).
However, interrogating the inhibition of PHD1 in a cell-based assay is complicated by the ubiquitous expression of PHD2. In this study, this was overcome by the generation of a knock-out PHD2 cell line using Zinc-finger nuclease technology. The choice of cell line was made after the relative expression of PHDs was measured in a range of cell lines and HepG2 cells were found to have high expression of PHD1 and PHD2 but very low levels of PHD3. The knock-out cell line could therefore be used to measure, more specifically, the inhibition of PHD1 by monitoring the compound concentration-dependent stabilization and accumulation of HIF1-alpha.

**Methods:** The quantification of inhibition of PHD1 and PHD2 in a recombinant enzyme assay was performed as described previously (5). For the cell-based assay, the cells were seeded overnight in full medium in the presence of inhibitor (16hours). The activity of compound was quantified by measuring the concentration-dependent stabilization and accumulation of HIF1-alpha using an MSD plate-based assay (6)

**Results:**

| | **Bayer 293 (non-selective)** | **Fibrogen 455 (selective)** | **Fibrogen 127 (selective)** |
|---|---|---|---|
| Recombinant Enzyme | $IC_{50}$ | | |
| PHD1 | 6nM | 100nM | 160nM |
| PHD2 | 8nM | 2100nM | 2800nM |
| Cell-based Assay | Activity | | |
| wild-type HepG2 | Active | Inactive | Inactive |
| PHD2 -/- HepG2 | Active | Active | Active |

**Conclusion:** The use of the PHD2-/- cell-line removes the effect of PHD2 from ablating the assay readout and thus obscuring the apparent efficacy of PHD1-selective compounds in a cell-based assay.

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