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Abstract 32

Discovery and Characterization of Novel, Potent, and Selective Hypoxia-Inducible Factor (HIF)-2α Inhibitors

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OVERVIEW

- Preclinical and clinical evidence suggests that HIF-2α inhibition is a valid approach to destroy tumor cells, particularly in clear cell renal carcinoma (ccRCC)[1,2].
- Arcus Biosciences is developing novel HIF-2α-specific small-molecule inhibitors and investigating the biology of HIF-2α in various cancer and non-cancer cell subsets.
- Here we describe the application of a pharmacophore mapping and structure-based design approach to discover multiple novel series of HIF-2α inhibitors which are characterized via a collection of in vitro assays. Highly optimized inhibitors exhibit low-nanomolar potency against HIF-2α and a favorable pharmacokinetic profile.

HIF-2α BIOLOGY & REGULATION

- The solid tumor microenvironment (TME) can be hypoxic and cancer cells require induction of genes associated with metabolism, proliferation, and angiogenesis to survive and metastasize[3].
- The master transcriptional regulators of hypoxia-induced genes are the Hypoxia-Inducible Factor (HIF) proteins[4].
- HIF consists of an oxygen-regulated alpha monomer, of which there are three isoforms (HIF-1α, HIF-2α, and HIF-3α)[4].
- Alpha monomers heterodimerize with a constitutively-expressed beta monomer (HIF-1β/ARNT) using Per-ARNT-SIM (PAS) protein-protein interaction domains[4].
- Disruption of HIF-α/HIF-1β heterodimer formation is an effective means to inhibition of HIF-2α-dependent gene transcription[4].

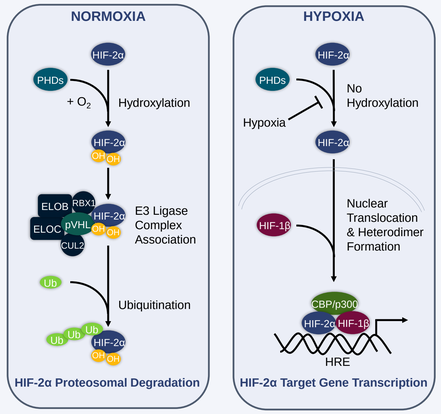


Figure 1. **Overview of HIF-2α regulation.** In normoxia (left), proline residues present in the oxygen-dependent degradation domain (ODDD) of HIF-2α are hydroxylated by prolyl hydroxylases (PHDs), allowing for recognition by the von Hippel-Lindau (pVHL) E3-ubiquitin ligase complex and subsequent ubiquitination and proteasomal degradation. Upon exposure to low oxygen conditions (hypoxia, right) or in the case of *vhl* mutation or silencing (pseudohypoxia), HIF-2α subunits accumulate and dimerize with HIF-1β/ARNT, resulting in transcription of various gene sets, some of which are pro-tumorigenic, downstream of hypoxia-response element (HRE) DNA binding sites. Adapted from Yu *et al.*[5].

INITIAL DESIGN, OPTIMIZATION, AND CHARACTERIZATION OF ARCUS HIF-2α INHIBITORS

Fundamentals of Targeting the HIF-2α/ARNT Complex

Small molecules have been designed to inhibit HIF-2α/ARNT heterodimerization by binding a small, internal cavity in the HIF-2α PAS-B domain. This hydrophobic cavity (shown as blue slate surface below) is fully enclosed with a volume of 290Å^3, and is occupied by 8 water molecules in the apo form. It has been demonstrated that small molecules can enter the cavity and induce a subtle conformation change of the HIF-2α PAS-B domain, which, in turn, results in destabilization of the HIF-2α/ARNT complex.[6]

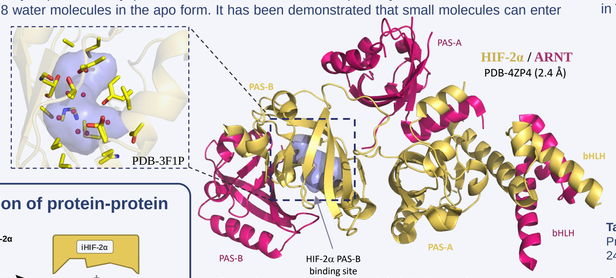
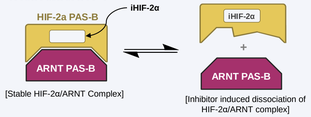


Figure 2. X-ray structure of HIF-2α/ARNT complex.

- **Basis for regulation of protein-protein interaction[6]:**



- **Small molecule binds to HIF-2α PAS-B cavity**
  - → Conformational change
  - – HIF dimerization disrupted
  - → Gene transcription inactive

- **Inhibitor design challenges:**
  - – Small internal pocket limits ligand size
  - – Binding affinity may not correlate with functional activity
  - – High affinity ligands often possess undesirable physicochemical properties (high lipophilicity)

Extensive Characterization of Initial HIF-2α Initial Lead Series

Assay	Series 1	Series 2	Series 3
HIF-2α 786-O Luc. (Cellular) IC_{50} (μM)	2.4	1.12	0.72
786-O Reporter (control, μM)	> 50	> 50	> 50
HIF-2α TSA $T_M\Delta$ (degrees)	5.1	5.3	6.3
HIF-2α MST K_D (μM)	1.5	0.44	0.13
HIF-2α ITC K_D (μM)	0.44	1.25	0.66
HIF-2α SPA IC_{50} (μM)	1.0	0.96	0.49

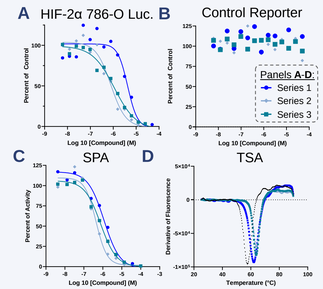


Table 1. Representative initial lead examples for Arcus Series 1, Series 2, and Series 3 HIF-2α inhibitor compounds. (MST = microscale thermophoresis, ITC = isothermal calorimetry) **Figure 3. A & B)** HIF and Control Cellular Reporter Assay. 786-O renal adenocarcinoma cells (mutant for VHL and HIF-1α) stably expressing HIF or control CMV luciferase reporter constructs (Qiagen) were treated with Arcus compounds for 20 hours (h) at 37°C 5% CO_2. **C)** Scintillation Proximity Assay (SPA). 50 nM PAS-B was incubated at room temperature with Arcus compounds in 2% DMSO for 60 min and 3 μg copper chelate PVT SPA beads for an additional 45 min before to addition of 25 nM ^{3}H-tracer and luciferase measurement. **D)** Thermal Shift Assay (TSA). Arcus compounds were incubated with PAS-B prior to addition of dye and fluorescence measurement. ΔT_m was calculated by normalizing compound T_m to DMSO T_m. Dotted lines, DMSO only.

Optimization of Series 1 HIF-2α Inhibitors

Series 1 inhibitors were optimized to improve potency and pharmacokinetic properties via structure-based design and iterative interpretation of structure activity relationships. A selection of optimized advanced prototypes are shown in Table 2 which potently inhibit HIF-2α function in numerous assays formats without appreciable off-target activity.

Assay	Arcus Compound 1	Arcus Compound 2	Arcus Compound 3	Arcus Compound 4	MK-6482 (PT2977, *competitor*)[7]
HIF-2α 786-O Luc. IC_{50} (nM)	61.5	9.4	15.5	4.2	22.8
786-O Reporter (control, nM)	>10,000	>10,000	>10,000	>10,000	> 10,000
HIF-2α SPA IC_{50} (nM)	64.0	22.7	14.9	14.6	30.4
VEGF Secretion IC_{50} (nM)	n.d.	75.9	51.1	21.1	93.1

Table 2. Potency of select series 1 inhibitors. HIF/control reporter and SPA assays performed as described in Table 1. VEGF Protein Secretion Assay - 786-O cells were treated with inhibitors for 48 hours at 37 °C 5% CO_2 (Media replaced with fresh after 24 hr). VEGF in the cell supernatant was quantified by AlphaLISA (Perkin Elmer).

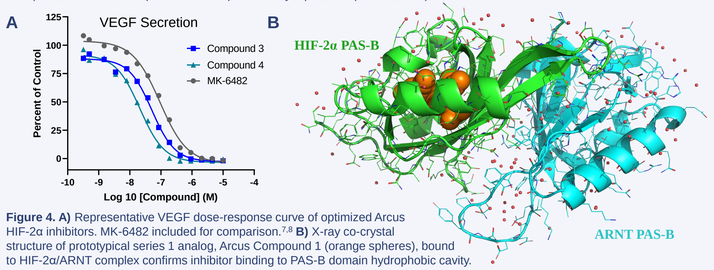


Figure 4. A) Representative VEGF dose-response curve of optimized Arcus HIF-2α inhibitors. MK-6482 included for comparison.[7,8] **B)** X-ray co-crystal structure of prototypical series 1 analog, Arcus Compound 1 (orange spheres), bound to HIF-2α/ARNT complex confirms inhibitor binding to PAS-B domain hydrophobic cavity.

Arcus Compound 3 Selectively Inhibits HIF-2α Gene Transcription

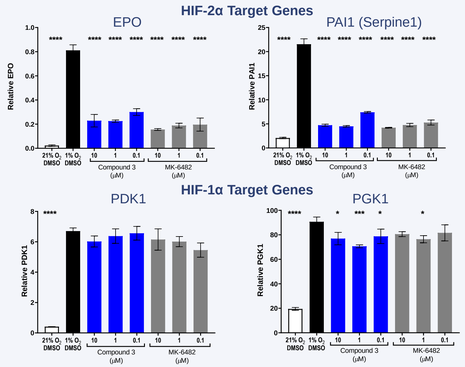


Figure 5. Prototype HIF-2α inhibitor (Compound 3) inhibits HIF-2α-, but not HIF-1α-, mediated transcription of pro-tumorigenic gene sets.

Hep3B hepatocellular carcinoma cells (wild-type for VHL and HIF-1α) were treated with 0.1, 1.0 , or 10 μM of Compound 3 or MK-6482 and exposed to hypoxia (1% O_2) for 16 h prior to RNA isolation. Gene expression levels of HIF-2α target genes (EPO and PAI1) and HIF-1α genes (PDK1 and PGK1) were determined by qPCR ($2^{-\Delta Ct}$ method) relative to HPRT1.

PHARMACOKINETIC PROFILING

Pharmacokinetic Characterization of Compound 3

Our advanced prototype HIF-2α inhibitor, Compound 3, exhibited a favorable in vitro pharmacokinetic profile with low intrinsic clearance in dog and human hepatocytes (Table 3). Furthermore, Compound 3 exhibited negligible inhibition against a panel of CYP isoforms (Table 4) and no time-dependent CYP inhibition (not shown). Compound 3 is further characterized by moderate-to-low clearance in rat and dog with high bioavailability in both species (Table 5).

Hepatocyte Stability

	Mouse	Rat	Dog	Human
$T_{1/2}$ (min)	21	100	340	950
CL_{int} (μL/min/10^6 cells)	33	6.9	2.1	0.7

Table 3. Summary of hepatocyte stability in various species.

CYP Isoform

	2C8	2C9	2C19	2D6	3A4
IC_{50} (μM)	>40	39.0	16.2	>40	>40

Table 4. Compound was evaluated in vitro for its potential to inhibit major human drug metabolizing enzymes of the cytochrome P450 family.

Preclinical Species Pharmacokinetics

Species	CL (L/h/kg)	Vss (L/kg)	$T_{1/2}$ (h)	F (%)
Rat	1.94	3.04	1.3	79
Dog	0.26	1.25	3.7	78

Table 5. Summary of experimental PK parameters in rat and dog. Rats were dosed 0.25 mg/kg IV in DMAC:Ethanol:Propylene Glycol:Saline (10:10:30:50) and 2 mg/kg PO in PEG400:Vitamin E TPGS (95:5). Dogs were dosed 0.33 mg/kg IV in DMA/PG/water (1:1:1) and 2 mg/kg PO in 1% HMPC.

SUMMARY

- Three distinct compound series are undergoing iterative SAR optimization to develop novel HIF-2α antagonists. Representative compounds from each series show both HIF-2α binding and functional activity in cell-based assays **(Table 1, Figure 3)**.
- Series 1 inhibitors have been optimized via structure-based design and interrogation of SAR trends to afford numerous potent advanced prototype HIF-2α inhibitors **(Table 2)**.
- A prototypical example, Compound 3, strongly inhibited HIF-2α target gene expression in Hep3B cells. In contrast HIF-1α target genes expression was minimally altered indicating Arcus inhibitors are highly selective for HIF-2α **(Figure 5)**.
- Optimized Arcus inhibitors, such as Compound 3, exhibit a favorable pharmacokinetic profile characterized by low intrinsic clearance in human hepatocytes and high oral bioavailability in preclinical species.

CITATIONS

1) Jonasch *et al.* (2020) ASCO 2020, Abstract #5003.
2) Srinivasan *et al.* (2020) ESMO, Abstract #LBA26.
3) Hockel & Vaupel (2001) JNCI 93, 266-276.
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5) Yu *et al.* (2019) Drug Disc Today 00, 1-9.
6) Rogers *et. al.* (2013) J Med Chem 56, 1739–1747
7) Data from Arcus test of molecule described in Wehn *et al.* (2018) J Med Chem 61, 9691-9721.
8) Xu *et. al.* (2019) J Med Chem 62, 6876–6893