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Discovery and characterization of AB521, a novel, potent, and selective hypoxia-inducible factor (HIF)-2α inhibitor

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ARCUS BIOSCIENCES

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) and tumors associated with mutant pVHL[1,2].
- Our group is developing novel specific small-molecule HIF-2α inhibitors and investigating the impact of HIF-2α modulation in tumor biology.
- 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].

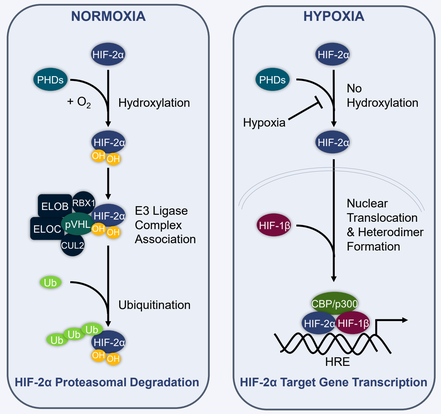


Figure 1. Overview of HIF-2α regulation. In normoxia (left), proline residues present in the oxygen-dependent degradation domain (ODDD) of HIF-2α are hydroxylated by prolyl hydroxylases (PHDs), allowing for recognition by the von Hippel-Lindau (pVHL) E3-ubiquitin ligase complex and subsequent ubiquitination 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 NOVEL 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]

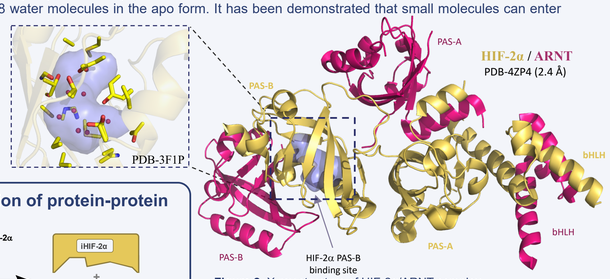
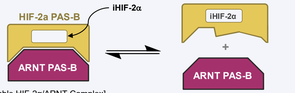


Figure 2. X-ray structure of HIF-2α/ARNT complex.

- **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)

Identification of Potent and Selective HIF-2α Inhibitors

Initial leads, exemplified by Compound A, were identified via pharmacophore mapping and structure-based design. Taken together, X-ray co-crystallization and thermal shift assays demonstrated avid binding to the PAS-B domain of the HIF-2α/ARNT complex and conformational perturbation of key residues (Y278, Y281, M252, and H293; Figure 3B) within the ligand binding pocket. From this lead, inhibitors were further optimized to improve potency and pharmacokinetic properties via structure-based design and iterative interpretation of structure activity relationships.

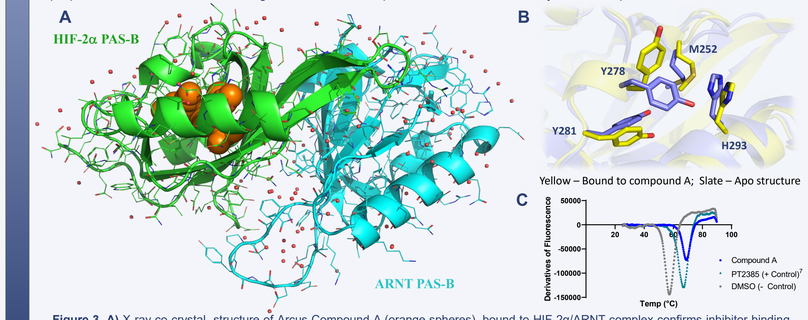


Figure 3. A) X-ray co-crystal structure of Arcus Compound A (orange spheres), bound to HIF-2α/ARNT complex confirms inhibitor binding to PAS-B domain hydrophobic cavity. **B)** Binding of Compound A (omitted for clarity) substantially alters the conformation of the HIF-2α/ARNT hydrophobic pocket, including key residues shown to disrupt HIF-2α/ARNT dimerization.[9] **C)** Thermal Shift Assay (TSA). Compounds were incubated with PAS-B prior to addition of dye and fluorescence measurement. ΔT_m (Cmpd A) = 11.6 °C

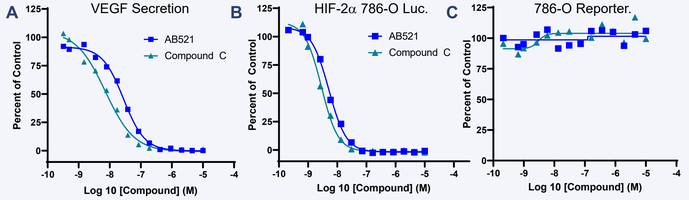
Characterization of Optimized HIF-2α Inhibitors

Further optimization led to the discovery of AB521, a highly potent and selective HIF-2α inhibitor. AB521 avidly binds to the HIF-2α PAS-B domain and potently inhibits HIF-2α function as evidenced by biochemical and cell-based assays.

Assay	Compound B	Compound C	AB521	MK-6482 (PT2977)[7]
HIF-2α 786-O Luc. IC_{50} (nM)	3.50 ± 0.91	3.56 ± 2.0	10.0 ± 3.7	15.9 ± 7.4
786-O Reporter (control, nM)	>10,000	>10,000	>10,000	> 10,000
HIF-2α SPA IC_{50} (nM)	n.d.	n.d.	19.1 (n=1)	31.2 ± 9.7
VEGF Secretion IC_{50} (nM)	9.61 ± 3.4	12.3 ± 0.14	30.5 ± 3.7	66.7 ± 37

Table 1. Potency and selectivity of optimized 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). (n ≥ 2 for all assays, unless noted otherwise)

Figure 4. Representative dose-response curves for VEGF secretion and HIF cellular reporter assays in 786-O cells.



AB521 Selectively Inhibits HIF-2α Gene Transcription

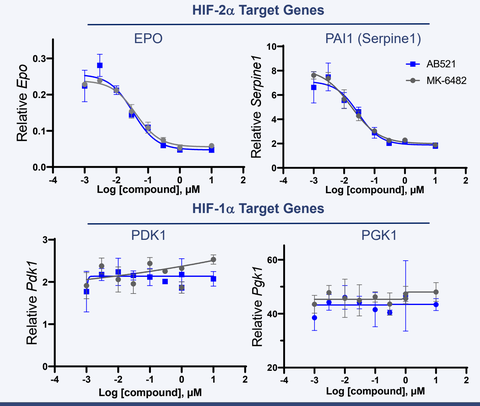


Figure 5. AB521 inhibits HIF-2α-, but not HIF-1α- mediated transcription of pro-tumorigenic gene sets. Hep3B hepatocellular carcinoma cells (wild-type for VHL and HIF-1a) were treated with 1 nM to 10 μM AB521 or MK-6482 and exposed to hypoxia (1% O_2) for 16 h prior to RNA isolation. Gene expression 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. AB521 inhibited EPO and PAI1 product with IC_{50} values of 33 and 28 nM, respectively.

PHARMACOKINETIC PROFILING

Pharmacokinetic Characterization of AB521

AB521 exhibited a favorable in vitro profile with low intrinsic clearance in dog and human hepatocytes (Table 3). Furthermore, AB521 exhibited negligible inhibition against a panel of CYP isoforms (Table 2) and no time-dependent CYP inhibition (not shown). AB521 is further characterized by moderate-to-low clearance in rat and dog (Table 3). AB521 is projected to a long half-life and low clearance in humans and is expected to be suitable for once-daily dosing.

CYP Isoform

	2C8	2C9	2C19	2D6	3A4
IC_{50} (μM)	>40	35.2	28.4	>40	>40

Table 2. Compound was evaluated in vitro for its potential to inhibit major human drug metabolizing enzymes of the cytochrome P450 family.

Pharmacokinetic Properties of AB521

Species	*Hepatocytes* CL_{int} (μL/min/10^6 cells)	*Hepatocytes* $T_{1/2}$ (h)	*In vivo* CL (L/h/kg)	*In vivo* Vss (L/kg)	*In vivo* $T_{1/2}$ (h)
Mouse	2.7	10.8	1.22	2.2	1.4
Rat	2.8	10.3	0.91	2.3	2.2
Dog	<0.7	>40	0.05	1.1	16
Human	<0.7	>40	0.012[a] *projected*	0.86[a] *projected*	50[a] *projected*

Table 3. 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). Dogs were dosed 0.33 mg/kg IV in DMA/PG/water (1:1:1). [a]Projected human PK parameters determined by allometry (mouse, rat, and dog). V_{ss} determined by Øie-Tozer method.

SUMMARY

- Novel leads, such as Compound A were demonstrated to avidly bind to the HIF-2α/ARNT complex and impart sufficient conformational perturbation to result in function disruption of HIF-2α/ARNT dimerization. **(Figure 3)**.
- Advanced prototype Compounds **B-C** and AB521 were discovered via structure-based design and interrogation of structure activity relationships. AB521 has been extensively characterized and found to be a potent and selective inhibitor of HIF-2α **(Table 1)**.
- AB521 strongly inhibited HIF-2α target gene expression in Hep3B cells. In contrast, HIF-1α target gene expression was minimally altered indicating our inhibitors are highly selective for HIF-2α **(Figure 5)**.
- AB521 exhibits a favorable pharmacokinetic profile characterized by low clearance in preclinical species and is projected to possess a pharmacokinetic profile in human suitable for once-daily dosing.

CITATIONS

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