Discovery and Optimization of 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) and tumors associated with mutant pVHL.[1,2]
- Our team has discovered a new series of HIF-2α inhibitors based on cycloalkylpyrazoles and performed their structure-activity relationship optimization study.
- Herein we present our key SAR findings and comprehensive pharmacology/DMPK characterization of top HIF-2α inhibitors in cycloalkylpyrazole series.

HIF-2α BIOLOGY & REGULATION

- Cancer cells adaptation to tumor hypoxic microenvironment requires induction of hypoxia response element (HRE) genes in response to oxygen shortages resulting in disease progression via increased angiogenesis, proliferation and metastasis.
- The hypoxic response is mediated transcriptionally via Hypoxia-Inducible Factor (HIF) proteins consisting of oxygen regulated HIF-1α, HIF-2α, and HIF-3α isoforms that heterodimerize with corresponding constitutively-expressed beta monomer (HIF-1β/ARNT) followed by nuclear translocation and expression of HRE genes.[3]
- The small-molecule inhibition of HIF-2α transcriptional activity via disruption of HIF-2α/ARNT transcription dimer complex formation is an effective cancer treatment strategy well established in animal models and clinical settings.[4]

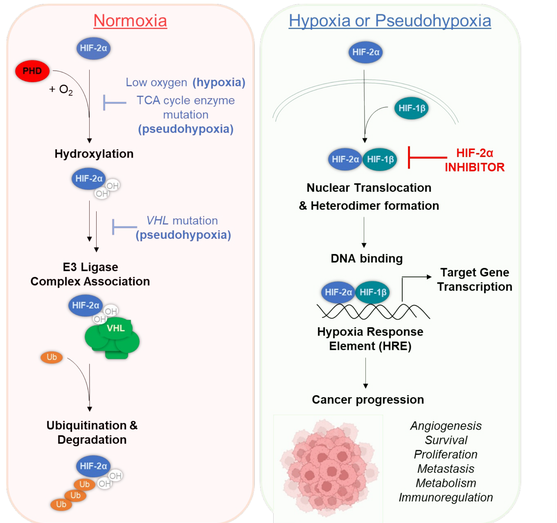


Figure 1. Overview of HIF-2α regulation. In normoxia (left), proline residues present in the oxygen-dependent degradation domain (ODDD) of HIF-2α are hydroxylated by prolyl hydroxylases (PHDs), allowing for recognition by the von Hippel-Lindau (pVHL) E3-ubiquitin ligase complex and subsequent ubiquitination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

DESIGN, OPTIMIZATION, AND CHARACTERIZATION OF NOVEL HIF-2α INHIBITORS

Initial Drug Design and Optimization

Our design strategy commenced with structural analysis of existing HIF-2α inhibitors and their key binding elements to HIF-2α PAS-B domain. Based on prevalence of HIF-2α inhibitors featuring 1-indanol moiety[6,7] we decided to perform replacement of this functional group with corresponding [6,5] and [5,5]-cycloalkyl-pyrazoles, while preserving the α-fluorinated alcohol moiety responsible for forming hydrogen bonding network with H293, Y281 and water molecule previously observed for PT2385 (Figure 1).

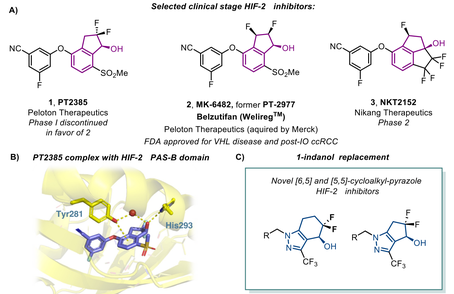


Figure 1. (a) Selected clinical stage HIF-2α inhibitors. (b) X-ray structure of HIF-2α with bound small molecule inhibitor PT2385 (PDB: 6E3S). Binding is facilitated by H293-[PT2385]-[H_2O]-Y281 hydrogen bonding network, Y281 π → PT2385 π* and phenoxide moiety hydrophobic interactions. (c) Initial structural design of cycloalkyl-pyrazole HIF-2α inhibitors.

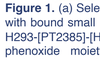
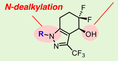
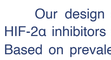
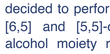
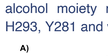
	HIF-2α Biochemical IC_{50} (nM)	HIF-2α Cell-Based IC_{50} (nM)*	HIF-2α Cell-Based 100% Serum IC_{50} (nM)	Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat
	>20,000	>10,000	>40,000	n.d.
	>20,000	>10,000	>40,000	n.d.
	4,850	4,610	>40,000	
	946	727	> 40,000	95 / 190
	164	154	4,780	
	89.2	59.2	736	260 / 110
	44.1	116	965	270 / 160

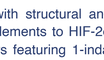
Table 1. The initial SAR profiling of pyrazole N-substitution allowed for a rapid identification of lead N-fluoroalkylpyrazole derivatives. The poor metabolic stability observed for this series was attributed to CYP-mediated N-dealkylation and glucuronidation of cyclohexanol moiety.

*HIF-2α and Cellular Reporter Assay. 786-O renal adenocarcinoma cells (mutant for VHL and HIF-1α) stably expressing HIF-2α CMV luciferase reporter constructs (Qiagen) were treated with Arcus compounds for 20 hours (h) at 37°C 5% CO_2

Identification of Compounds with Improved Metabolic Stability



Compound ID	HIF-2α Cell-Based IC_{50} (nM)	HIF-2α Cell-Based 100% Serum IC_{50} (nM)	Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat
 4a	49.3	328	235 / 174
 4b	12.9	59.0	63 / 184
4c	>20,000	>40,000	n.d.
4d	164	217	37 / 192
4e	383	2,200	<2.7 / 32.2
4f	767	>40,000	<2.7 / 4.79
4g	1,670	>40,000	n.d.



Compound ID	HIF-2α Cell-Based IC_{50} (nM)	HIF-2α Cell-Based 100% Serum IC_{50} (nM)	Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat
5a	66.4	204	358 / 418
5b	20.1	97.9	98 / 226
5c	8,600	>40,000	n.d.
5d	42.7	111	17 / 87
5e	387	812	12.7 / 31.8
5f	86.6	628	70 / 27
5g	41.3	372	6.3 / 23.3

Table 2. Broad screening of N1-substitution of [5,6] and [5,5]-cycloalkyl-pyrazoles afforded compounds with improved metabolic stability featuring sulfone and varied polyfluorinated cycloexhane N1-substituents. [5,5]-cycloalkyl-pyrazole derivatives consistently demonstrated improved HIF-2α potency over their [5,6]-bicyclic counterparts.

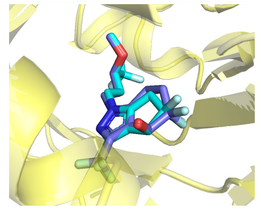


Figure 2. Overlay of X-ray structures for compounds **4b** and **5b** bound to HIF-2α PAS-B domain

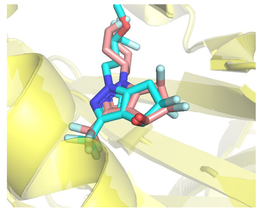
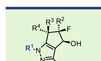


Figure 3. Overlay of X-ray structures for compounds **5b** and **7e** bound to HIF-2α PAS-B domain

Structural optimization of sulfone-containing [5,5]-cycloalkyl-pyrazole inhibitors



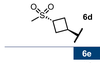
	R²	R³	R⁴	HIF-2α Cell-Based IC_{50} (nM)	HIF-2α Cell-Based 100% Serum IC_{50} (nM)	Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat
 6a	F	H	H	330	890	< 2.7 / 4.0
 6b	F	H	H	4,940	22,300	n.d.
 6c	F	H	H	1,930	5,980	n.d.
 6d	F	H	H	5,660	27,000	n.d.
6e	**F**	**H**	**H**	**208**	**411**	**5.04 / 46.8**
6f	H	H	H	680	1,450	<2.7 / 7.24
6g	F	F	H	354	1,050	n.d
6h	F	H	F	202	550	9.7 / 51.0
 6i	H	F	H	1,620	7,190	n.d

Table 3. Structural modification of [5,5]-cycloalkyl-imidazoles containing sulfone moiety. An extensive modification of polyfluorocyclopentanol fragment in compounds featuring the most promising thiethane dioxide fragment (**6e-i**) resulted in an identification of compound **6e** featuring adequate combination of HIF-2α potency and metabolic stability in this series.

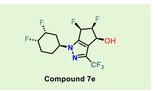
Fluorination pattern screening for N-cyclohexyl [5,5]-cycloalkyl-pyrazole inhibitors

	R²	R³	R⁴	HIF-2α Cell-Based IC_{50} (nM)	HIF-2α Cell-Based 100% Serum IC_{50} (nM)	Hepatocyte CL_{int} (μL/min/10^6 cells) hu / rat
 7a	F	H	H	174	1,240	11.5 / 17.5
 7b	F	H	H	253	1480	n.d.
 7c	F	H	H	6,300	584	n.d.
7d	F	H	H	154	39.2	5.2 / 67.2
7e	**H**	**F**	**H**	**52**	**190**	**< 2.7 / 3.3**
7f	F	H	F	430	7690	-
7g	F	F	H	12	42	13.2 / 15.1
7h	F	H	H	60	336	6.3 / 3.9
7i	**H**	**F**	**H**	**104**	**269**	**< 2.7 / < 2.7**
7j	H	H	F	581	5,360	-
7k	F	F	H	19	93	8.7 / 20.7
7l	F	H	F	87	667	-

Table 4. SAR study of N-cyclohexyl-[5,5]-cycloalkyl-imidazoles with diverse fluorination pattern. Incorporation of 3,4-*syn*-difluoro- and 3,4,5-*syn*-trifluorocyclohexane N-substituents in combination with *syn*-difluorination pattern of the core structure afforded compounds **7e** and **7i** that were selected for further characterization.

PHARMACOLOGY / DMPK CHARACTERIZATION OF INHIBITOR 7E

Compound 7e selectively Inhibits HIF-2α Gene Transcription



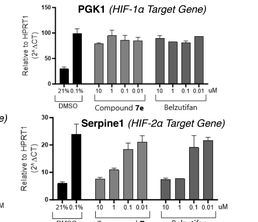


Figure 4. Compound **7e** inhibits HIF-2α-, but not HIF-1α-, mediated transcription of pro-tumorigenic gene sets. Hep3B hepatocellular carcinoma cells (wild-type for VHL and HIF-1α) were treated with 10 nM to 10 μM of **7e** or MK-6482 and exposed to hypoxia (1% O_2) for 16 hours. Gene expression levels of HIF-2α target genes (EPO and PAI-1) and HIF-1α gene PGK1 were determined by qPCR relative to *HPRT1* ($2^{-\Delta Ct}$).

Pharmacokinetic Profiling of 7e in Preclinical Species

Compound 7e	In Vitro (Hepatocytes)		In vivo		
Species	CL_{int} (μL/min/10^6 cells)	$f_{u,p}$	CL (L/h/kg)	Vss (L/kg)	$T_{1/2}$ (h)
Mouse	**33.5**	0.443	**2.13**	1.59	1.56
Rat	**3.20**	0.597	**1.25**	3.1	2.71
Dog	**5.40**	0.576	**0.740**	0.98	1.55

Table 5. Compound **7e** exhibits a favorable pharmacokinetic profile characterized by moderate-to-low clearance in preclinical species and is stable to human hepatocytes.

SUMMARY

- We have identified a new series of potent and isoform selective HIF-2α inhibitors based on the cycloalkylpyrazole scaffold. The SAR study was designed utilizing biochemical SPA assay and HIF-2α-dependent transcription in a HIF-2α-specific luciferase reporter transcription cell based (786-O cells) assay.
- Compounds based on a [5,5]-cycloalkylpyrazole core demonstrated broader tolerance to structural modification during the initial lead optimization in contrast to their [5,6]-bicyclic counterparts.
- Systematic approach towards analogs with decreased lipophilicity yielded potent HIF-2α inhibitors **6e**, **7e**, **7i**, characterized by low rate of metabolic degradation measured upon incubation with human and rat hepatocytes.
- Based on balanced combination of HIF-2α potency and metabolic stability, compound **7e** was selected for comprehensive pharmacology and ADME profiling. This compound demonstrated low DDI-potential in CYP inhibition assays, low in vivo clearance and excellent bioavailability in rodents. Additionally, compound **7e** selectively and efficaciously inhibited expression of HIF-2α target genes in Hep3B hepatocellular carcinoma cells.

REFERENCES

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7) Buchstaller (**2023**) *J. Med. Chem.*,66, 8666