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Discovery of Orally Bioavailable and Liver Targeted Hypoxia-Inducible Factor Prolyl Hydroxylase (HIF-PHD) Inhibitors for the Treatment of Anemia

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Discovery of Orally Bioavailable and Liver Targeted Hypoxia-Inducible Factor Prolyl Hydroxylase (HIF-PHD) Inhibitors for the Treatment of Anemia

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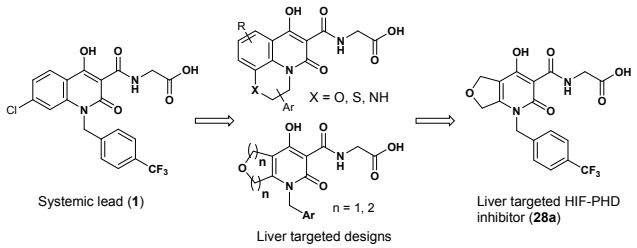


Abstract

We report herein the design and synthesis of a series of orally active, liver targeted hypoxia-inducible factor prolyl hydroxylase (HIF-PHD) inhibitors for the treatment of anemia. In order to mitigate the concerns for potential systemic side effects, we pursued liver-targeted HIF-PHD inhibitors relying on uptake via organic anion transporting polypeptides (OATPs). Starting from a systemic HIF-PHD inhibitor (**1**), medicinal chemistry efforts directed toward reducing permeability and at the same time maintaining oral absorption led to the synthesis of an array of structurally diverse hydroxypyridone analogs. Compound **28a** was chosen for further profiling because of its excellent in vitro profile and liver selectivity. This compound significantly increased hemoglobin levels in rat following chronic QD oral administration and displayed selectivity over systemic effects.

Key Words

Anemia, HIF-PHD inhibitors, EPO, hydroxypyridone, OATP, permeability, liver selective

Anemia is a disease condition where patients have lower than normal hemoglobin (Hb) levels in the blood, usually < 12 g Hb/dL.[1] Anemic patients experience fatigue, shortness of breath and diminished functional status, and therefore endure suboptimal quality of life. The common causes of anemia include a range of medical conditions, such as chronic kidney disease (CKD),[2] chemotherapy induced anemia (CIA),[3] and anemia of chronic disease (ACD).[4,5] Currently, the standard of care for anemia seeks to enhance patient functionality and quality of life by restoring effective red blood cell (RBC) production through parenteral administration of recombinant human erythropoietin (rhEPO).[6] Despite significant advantages over RBC transfusions, the treatment of anemia with EPO analogs suffers from many shortcomings, including inconvenience, EPO resistance, and most importantly, safety concerns. It has been known that rhEPO treatment may contribute to adverse cardiovascular outcomes.[7] Therefore, the industry has continued efforts toward new treatments for anemia.

Accumulating evidence has suggested that hypoxia-inducible factor prolyl hydroxylase (HIF-PHD) inhibitors act as hypoxia mimetics by stabilizing the transcription factor HIF, which modulates numerous genes, including those involved in erythropoiesis and iron-handling.[8] Proof-of-concept for the treatment of anemia in patients with CKD has been achieved for this mechanism with Fibrogen's small molecule HIF-PHD inhibitors FG-2216[9] and FG-4592.[10] To date at least six small molecule HIF-PHD inhibitors have been tested in humans for treating renal disease-associated anemia.[11-13] Recently, we also disclosed two structurally diverse HIF-PHD inhibitors.[14,15] To our knowledge, all of these compounds are systemic, non-tissue selective HIF-PHD inhibitors. Given the complex pharmacology of HIF stabilization and its broad tissue expression,[16] systemic HIF-PHD inhibition may have pleiotropic effects. In order to mitigate the potential risk for undesired systemic effects, we pursued a liver-targeted HIF-PHD inhibitor.[17] Herein we report our efforts toward this end.

During fetal development, liver is the primary organ producing EPO; postnatally, EPO is mainly produced by kidney and, to a lesser extent, by liver.[18] Then, through systemic circulation, EPO reaches bone marrow where it binds to the EPO receptor, stimulating erythropoiesis. We hypothesized that liver selective HIF-PHD inhibition would "re-activate" hepatic EPO production, triggering sufficient RBC generation to ameliorate anemia, while avoiding potential systemic side effects, such as pulmonary arterial pressure (PAP) increase (Figure 1).[19] Indeed, Minamishima and Kaelin has shown that liver-selective deletion of PHD1/2/3 in mice resulted in increased serum EPO levels that paralleled the mRNA levels in liver and exceeded those achieved by renal PHD2 knockout.[20] Consistent with the hypothesis, liver selective knockdown with PHD2 siRNA in rhesus led to clinically relevant increases in hemoglobin.[21] In

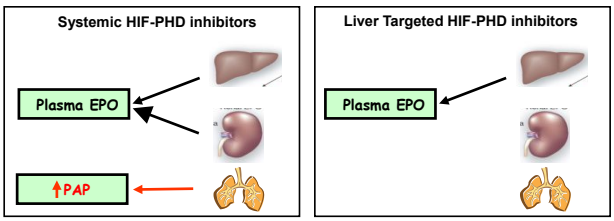


Figure 1. Hypothesis: effects of HIF-PHD inhibition - systemic vs liver targeted.

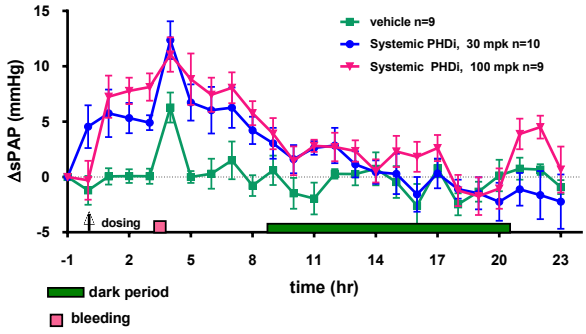


Figure 2. Systolic PAP increase with systemic HIF-PHDi FG-2216 in PAP telemetry rats.

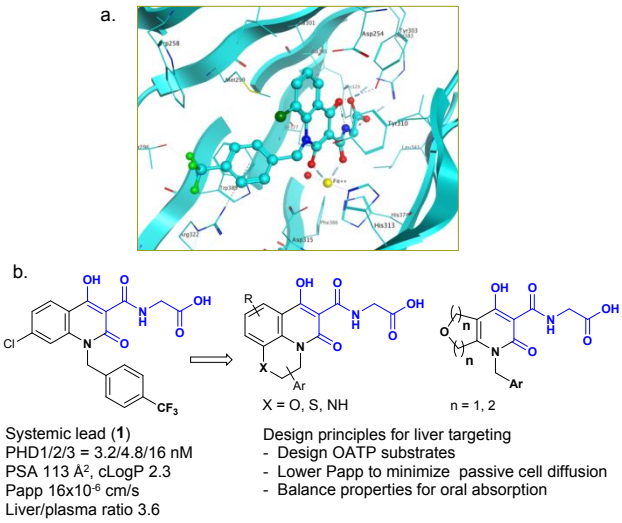


Figure 3. a) X-ray co-crystal structure of a PHD2 domain with Cl regioisomer of **1**; b) From systemic lead to hepatoselective designs.

addition, we have demonstrated that a systemic HIF-PHD inhibitor (HIF-PHDi) had increased systolic PAP (sPAP) in a dose dependent manner in a telemetered rat model (Figure 2).[22]

With this supporting evidence in mind, we undertook a discovery effort to identify a hepatoselective HIF-PHD inhibitor. There existed several approaches for liver targeting, for example, utilization of liver-specific transport proteins,[23] HepDirect cytochrome P450-activated prodrugs,[24] and nanoparticles to deliver incorporated therapeutic agents.[25] We decided to design substrates for active transport relying on organic anion transporting polypeptides (OATPs)[23] to achieve hepatoselectivity, and strategically envisioned meeting this objective through modification of a systemic lead (**1**).[26] Specifically, we sought to keep the critical structural features (highlighted in blue in Figure 3) intact

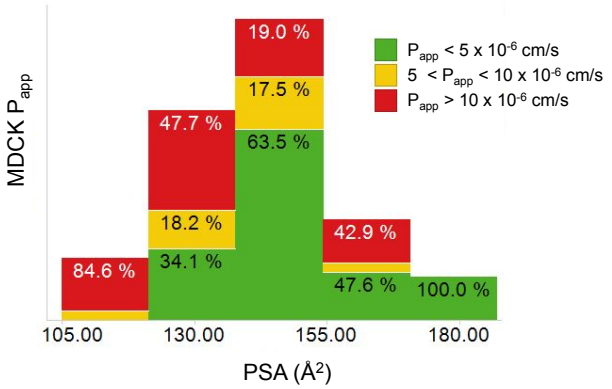


Figure 4. PSA > 130 Å^2 increases probability of desired permeability (Papp < 5 x 10-6 cm/s).

to maintain key interactions with Fe^{2+}, Tyr303, and Arg383 of the enzyme, and study the SAR of the other parts of the molecule following the design principles outlined in Figure 3.

Our SAR studies focused on optimizing liver selectivity and balancing properties to achieve acceptable oral absorption. Our goal was to increase liver selectivity by engaging active transport into hepatocytes via the liver specific OATPs, and by simultaneously decreasing the extent of passive cell permeability. Acidic moieties can serve as key transporting elements to enable recognition by the OATP transport proteins. Our strategy was to take advantage of the carboxylic acid group present in the systemic lead and design away from passive transport.

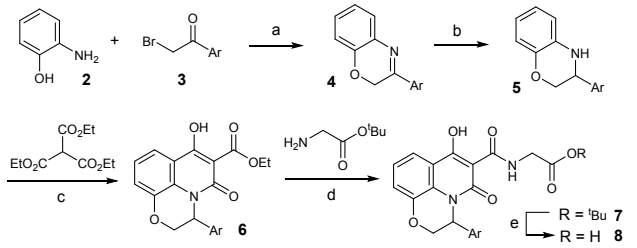
There are three isoforms of PHD, namely, PHD1, PHD2, and PHD3, and inactivation of all the three isoforms leads to optimal erythrocytosis.[12] We aimed to develop a pan-PHD inhibitor. After assessment of compounds in assays for PHD catalytic activity, compounds of interest were selected for passive permeability (P_{app}) evaluation. Compounds with low permeability (P_{app} < 10 x 10^{-6} cm/s) were further evaluated in rat tissue PK where the distribution ratio of liver vs plasma was obtained.[22]

Passive permeability is closely related to molecular properties such as lipophilicity (LogP)[27] and polar surface area (PSA).[28] In order to understand the relationship between the calculated molecular properties (cLogP and PSA) and hepatoselectivity for this hydroxypyridone lead series, and use the former as a prediction of the latter to enable prospective designs, we assembled a list of compounds that spanned a range of lipophilicity (cLogP = 0.5 – 4) and polar surface area (PSA = 105 – 180) and measured their P_{app}. As can be seen from Figure 4, PSA > 130 Å^2 increased probability of desired permeability (Papp < 5 x 10-6 cm/s), and in general, PSA > 130 Å^2 correlated to cLogP < 3 for these compounds. Therefore, in silico calculation of PSA and cLogP was used for prioritizing targets.

The synthesis of the hydroxypyridone analogs for SAR studies is described in Schemes 1-4.[22] Reaction between aminophenol **2** and α-bromo ketone **3** provided cyclic imine **4**. Upon reduction, the resulting aniline **5** was condensed with triethylmethanetricarboxylate to afford tricyclic core **6**. Amide formation with *tert*-butyl glycinate followed by hydrolysis of the ester completed the synthesis of tricyclic analogs **8** (Scheme 1).[29]

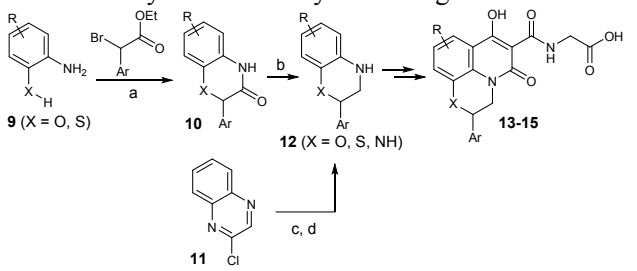
Similarly, the regioisomeric tricyclic analogs **13** and **15** were synthesized starting with cyclization between substituted aniline **9** and an α-bromo ester to provide lactam **10** (Scheme 2). Reduction of amide **10** gave

Scheme 1. Synthesis of tricyclic analogs **8**[a]



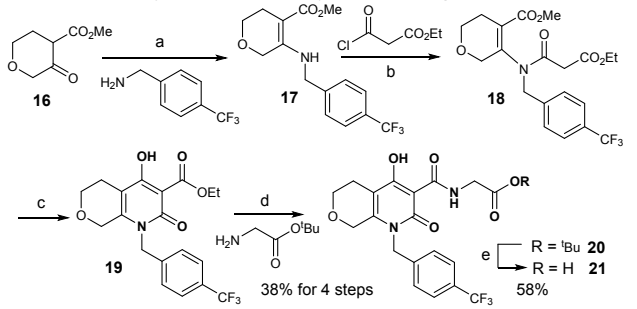
[a]Reagents and conditions: (a) Bu_4NHSO_4, aqueous K_2CO_3, dichloromethane; (b) $NaBH_3CN$, acetic acid, dichloromethane/methanol; (c) triethyl methanetricarboxylate, 200 °C; (d) $^iPr_2EtN$, toluene, reflux; (e) TFA, dichloromethane.

Scheme 2. Synthesis of tricyclic analogs **13-15**[a]



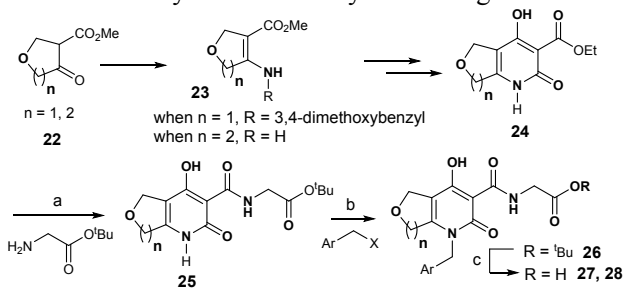
[a]Reagents and conditions: (a) Base, toluene, 120 °C; (b) BH_3-Me_2S, THF, 0 °C – rt; (c) (6-(trifluoromethyl)pyridin-3-yl)boronic acid, $Pd(Ph_3P)_4$, K_2CO_3, dioxane/water, 90 °C; (d) BH_3-THF, THF, rt.

Scheme 3. Synthesis of bicyclic analog **21**[a]



[a]Reagents and conditions: (a) Ethanol, acetic acid, 90 °C; (b) CH_3CN, 70 °C; (c) NaH, toluene/ethanol; (d) DME, $^iPr_2EtN$, 90 °C; (e) TFA, dichloromethane.

Scheme 4. Synthesis of bicyclic analogs **27** and **28**[a]



[a]Reagents and conditions: (a) IPA, 100 °C; (b) K_2CO_3, acetone, 40 °C; (c) TFA, dichloromethane.

cyclic aniline **12** (X = O or S). For compound **14**, intermediate **12** (X = NH) was obtained through another two step sequence: Suzuki coupling and borane reduction. Finally, intermediate **12** was converted to **13-15** following the same procedures described for **8**.

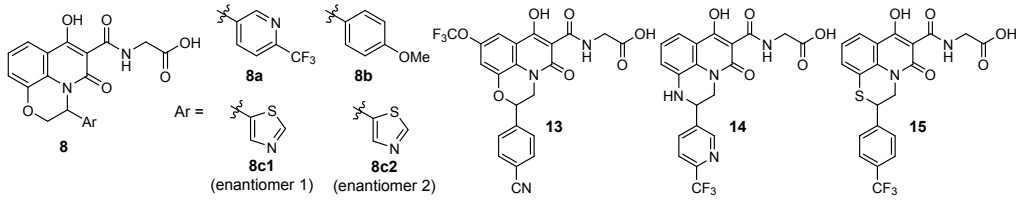
Scheme 3 illustrates the synthesis of bicyclic analog **21**. β-Keto ester **16** was condensed with *p*-CF_3-benzylamine to afford enamine **17**. Acylation of **17** gave compound **18** which was engaged in a Dieckmann type reaction to arrive at core structure **19**.[30] Finally, amide formation and hydrolysis of the *tert*-butyl ester provided bicyclic analog **21**.

While the synthetic route in Scheme 3 would allow access to many bicyclic analogs described herein, an efficient synthesis to rapidly explore the SAR of the benzyl portion was needed. As illustrated in Scheme 4, pyridone **24** was synthesized via enamine **23** as a key intermediate. After amide formation with *tert*-butyl glycinate, advanced intermediate **25** provided access in 2 steps, including alkylation and ester hydrolysis, to final products **27** and **28** with diverse benzyl groups.[31,32]

For the engagement of OATPs, we took advantage of the carboxylic acid moiety of lead compound **1**, and designed a series of tricyclic analogs to probe liver selectivity. As shown in Table 1, diverse structural features were tolerated in terms of potency, where the third ring could be a morpholine (**8** and **13**), piperazine (**14**) or thiomorpholine (**15**), and the aromatic group (Ar) could be attached at either position of the ethylene (**8** vs **13** – **15**). However, despite that calculated PSA was >130 Å^2 (except for compound **15**) and clogP < 3, which enriched measured P_{app} of < 5 x 10^{-6} cm/s, these tricyclic analogs exhibited suboptimal liver/plasma ratios in rat (<8).

We therefore focused our efforts on the bicyclic series where a tetrahydropyran or tetrahydrofuran ring is fused with the hydroxypyridone (Table 2). Again, the analogs with PSA > ~130 Å^2 and clogP < 3 were prioritized for synthesis. Several trends were observed. The bicyclic analogs exhibited much higher liver selectivity than the tricyclic analogs despite similar PSA, cLogP and P_{app} values. Within the bicyclic series, one pyran regioisomer was significantly more

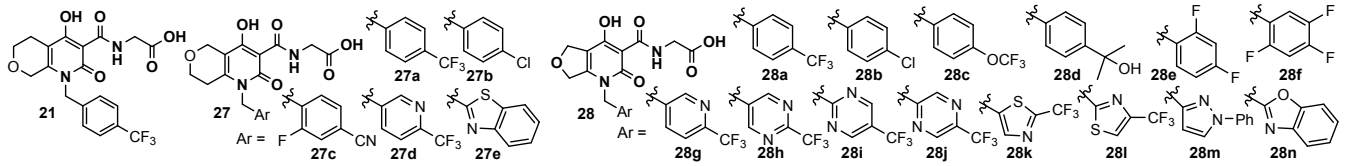
Table 1. SAR study of the tricyclic analogs



Cmpd	hPHD1[a] IC_{50} (nM)	hPHD2[a] IC_{50} (nM)	hPHD3[a] IC_{50} (nM)	PSA[c] (Å^2)	cLogP[d]	Papp (10^{-6} cm/s)	Liver / Plasma ratio[e]
8a	4.9 ± 3.3	6.1 ± 4.2	8.9 ± 3.8	140	-1.1	3.1	1.0
8b	27 ± 14	27 ± 4.0	21 ± 5.8	139	-0.2	7.4	6.2
8c1	7.4 ± 1.4	15 ± 3.5	nd[b]	143	-1.9	< 2.3	2.5
8c2	2.1 ± 0.6	4.2 ± 0.4	nd[b]	143	-1.9	< 1.8	4.4
13	1.4 ± 0.3	2.0 ± 0.3	5.1 ± 0.1	165	-0.5	nd[b]	7.9
14	2.3 ± 0.3	5.4 ± 1.2	6.6 ± 0.2	142	-1.4	nd[b]	3.9
15	3.8 ± 1.2	6.5 ± 0.5	13 ± 2.7	115	1.7	< 8.6	7.5

[a]Human HIF-PHD IC_{50} data expressed as mean ± SD (n≥2 independent experiments). [b]Not determined. [c]PSA was calculated using the TPSA method published in J. Med. Chem. 2000, 43, 3714-3717. [d]cLogP was calculated at pH 7.4 using ACD Percepta software. [e]Total liver/plasma ratio at 4 hr (10 mg/kg PO).

Table 2. SAR study of the bicyclic analogs



Cmpd	hPHD1[a] IC_{50} (nM)	hPHD2[a] IC_{50} (nM)	hPHD3[a] IC_{50} (nM)	PSA[d] (Å^2)	cLogP[e]	Papp (10^{-6} cm/s)	Liver / Plasma ratio[f]
21	11 ± 2.1	20 ± 1.1	40 ± 5.6	128	0.9	7.3	10 (31)
27a	14 ± 7.3	19 ± 11	51 ± 3.9	128	1.1	4.0	107
27b	5.9 ± 0.5	8.5 ± 1.6	33 ± 4.2	128	1.2	3.6	216
27c	6.3 ± 0.2	9.0 ± 0.8	18 ± 1.1	152	-0.2	nd[b]	74
27d	6.8 ± 0.1	11 ± 3.3	41[c]	140	-0.4	<1.7	126
27e	3.3 ± 1.0	5.4 ± 3.2	14[c]	142	1.2	<2.1	217
28a	3.6 ± 1.8	4.9 ± 1.8	8.2 ± 3.3	126	1.0	4.2	16 (38)
28b	2.1 ± 0.1	2.3 ± 0.5	6.4 ± 2.4	130	1.0	2.5	39
28c	2.4 ± 0.7	3.1 ± 0.1	5.8[c]	139	1.3	5.2	22
28d	6.2 ± 2.1	6.9 ± 3.6	16 ± 4.7	149	-0.1	<1.5	210
28e	2.2 ± 0.4	2.6 ± 0.3	8.8 ± 5.0	129	0.5	< 2.2	50
28f	3.1 ± 1.4	4.4 ± 2.9	6.6[c]	129	0.6	nd[b]	87
28g	3.6[c]	5.3[c]	13[c]	142	-0.5	<1.7	166
28h	11 ± 1.3	6.8 ± 0.6	18 ± 6.2	157	-1.1	nd[b]	63
28i	12 ± 2.7	10 ± 0.2	22 ± 0.2	155	-0.7	nd[b]	48
28j	4.9 ± 2.6	4.9 ± 2.6	13 ± 3.2	154	-1.5	nd[b]	359 (952)
28k	2.4 ± 0.7	4.0 ± 0.6	6.4 ± 1.3	145	-0.1	< 1.5	212

28l	3.7 ± 1.0	4.3 ± 0.7	7.8 ± 0.5	143	0.5	nd[b]	587
28m	3.2 ± 1.3	3.8 ± 1.5	6.0 ± 1.5	143	0.3	nd[b]	69
28n	5.0 ± 2.1	4.2 ± 0.7	6.6[c]	156	0.6	< 1.9	60

[a]Human HIF-PHD IC_{50} data expressed as mean ± SD (n≥2 independent experiments) unless noted otherwise. [b]Not determined. [c]Measured only once. [d]PSA was calculated using the TPSA method published in J. Med. Chem. 2000, 43, 3714-3717. [e]cLogP was calculated at pH 7.4 using ACD Percepta software. [f]Total liver/plasma ratio at 4 hr post dose (10 mg/kg PO); unbound liver/plasma ratio in parentheses where available. In general, unbound fraction was higher in liver than in plasma, and unbound ratio was approximately 3x total ratio for this series of analogs.

liver selective than the other (**27a** vs **21**, liver/plasma ratio 107 vs 10), and furan analogs were more potent than their pyran counterparts (**28a** vs **21**, **27a**; **28b** vs **27b**; and **28g** vs **27d**). Finally, the in silico parameters PSA and cLogP were indeed a useful tool in predicting P_{app} for this chemical matter.

Our strategy to use a carboxylic acid moiety as the OATP recognition element and design away from passive transport was verified by HIF1α assay in MDCK and MDCK/hOATP1B1 cells (Figure 5).[22] Compound **28a**, a low permeability compound, did not show HIF1α activity in MDCK cells (blue line), but the activity was boosted in MDCK/hOATP1B1 cells (red line) suggesting **28a** was actively transported via a human OATP.

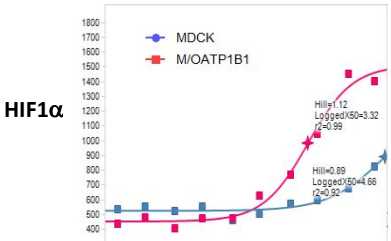


Figure 5. HIF1α assay of **28a** in MDCK and MDCK/hOATP1B1 cells.

One potential limitation of achieving liver selectivity by relying on OATPs and reduced permeability is that as a compound becomes less permeable, its oral absorption is typically impeded. Therefore, choosing the appropriate level of permeability is critical to balancing hepatoselectivity and oral absorption in a compound. Compound **28a** was selected for further profiling because of its appropriate permeability that led to a good hepatoselectivity (unbound liver:plasma = 38:1) as well as acceptable PK properties across species (Table 3).

Table 3. Pharmacokinetic profile of compound **28a**[a]

Species	Plasma Cl (mL/min/kg)	Vd (L/kg)	$t_{1/2}$ (h)	F (%)
Rat[b]	11	3.1	7.3	39
Dog[c]	17	5.6	6.2	25

[a]Vehicles for rat, *iv* and *po*: PEG200/HPCD/water (30:12:58, v/v/v). Dog, *iv*: PEG200/HPCD/water (30:12:58 v/v/v); *po*: Imwitor/Tween (1:1 w/w). [b]1 mg/Kg *iv*, 2 mg/kg *po*. [c]0.175 mg/kg *iv*, 1 mg/kg *po*.

An off-target screen of this compound was performed against a panel of 168 receptors, ion channels, and enzymes, and only one off-target activity was found with IC_{50} < 10 μM (leukotriene, cysteinyl $CysLT_2$: IC_{50} = 4.5 μM). Compound **28a** had no effects on cardiac ion channels (IC_{50} of iKr > 60 μM, Nav1.5 > 30 μM, Cav1.2 > 30 μM) and was also selective over CYP 3A4 (IC_{50} > 50 μM) and hPXR (EC_{50} > 30 μM, 1% Act).[33]

With the promising PK and selectivity profile, **28a** was further characterized pharmacodynamically. As shown in Figure 6, significant increase in hemoglobin was observed following a 4 week treatment of **28a** in rat (QD, PO) at 30 mpk (panel a), and based on this data, the minimal efficacious dose (MED) should be approximately 30 mpk. In the rat telemetry study (panel b), **28a** showed no effect on the pulmonary arterial pressure (PAP) in rat at 100 mg/kg, roughly 3x MED. On the other hand, systemic HIF-PHD inhibitor FG-2216 caused increase in PAP at 50 mpk. Therefore, these studies have demonstrated that liver-selective inhibition of HIF-PHD drives useful increases in hemoglobin separate from systemic side effects.

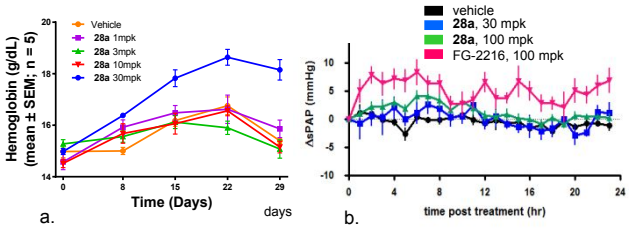


Figure 6. *In vivo* studies of **28a** in rat: a) chronic PD study where significant hemoglobin increase was observed at 30 mpk; b) rat telemetry study where no effect on PAP was observed at 3x MED.

In summary, we have designed and synthesized a series of bioavailable and liver targeted HIF-PHD inhibitors that may be used for the treatment of anemia. We relied on organic anion transporting polypeptides (OATPs) to achieve liver selectivity. A major focus of the optimization effort was to decrease the passive cell permeability to achieve hepatoselectivity, and simultaneously balance molecular properties to achieve acceptable bioavailability. These efforts led to the identification of compound **28a** that possessed excellent potency in vitro, liver selectivity and acceptable PK properties. This compound significantly increased hemoglobin in rat following chronic QD oral administration and displayed selectivity over systemic effects. Further optimization to improve overall PK profile will be reported in due course.

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Supporting Information Available. Synthetic procedures and characterization data of selected compounds, conditions for the biological assays, and protocol for pharmacokinetic and pharmacodynamic studies. This material is available free of charge via the Internet at http://pubs.acs.org.

Abbreviations: EPO, erythropoietin; rhEPO, recombinant human erythropoietin; HIF, hypoxia inducible factor; PHD, prolyl hydroxylase; RBC, red blood cell; Hb, hemoglobin; CKD, chronic kidney disease; CIA, chemotherapy induced anemia; ACD, anemia of chronic disease; PAP, pulmonary arterial pressure; OATP, organic anion transporting polypeptide; PSA, polar surface area; Papp, apparent permeability; MDCK, Madin Darby canine kidney; PO, oral; QD, once a day; MED, minimum effective dose; PXR, Pregnane X receptor.

References

(1) Beutler E.; Waalen J. The definition of anemia: what is the lower limit of normal of the blood hemoglobin concentration? *Blood* **2006**, *107*, 1747–1750.

(2) Vilayur, E.; Harris, D. C. H. Emerging therapies for chronic kidney disease: what is their role? *Nature Rev. Nephrol.* 2**009**, *5*, 375–383.

(3) Littlewood, T. J.; Collins, G. P. Pharmacotherapy of anemia in cancer patients. *Expert Rev. Clin. Pharm.* **2008**, *1*, 307–317.

(4) Cavill, I.; Auerbach, M.; Bailie, G. R.; Barrett-Lee, P.; Beguin, Y.; Kaltwasser, P.; Littlewood, T.; Macdougall, I. C.; Wilson, K. Iron and the anaemia of chronic disease: a review and strategic recommendations. *Curr. Med. Res. Opin.* **2006**, *22*, 731–737.

(5) Weiss, G.; Goodnough, L. T. Anemia of chronic disease. *N. Engl. J. Med.* **2005**, *352*, 1011–1023.

(6) Jelkmann, W. Developments in the therapeutic use of erythropoiesis stimulating agents. *Br. J. Haematol* **2008**, *141*, 287–297.

(7) Stohlawetz, P. J.; Dzirlo, L; Hergovich, N. Effects of erythropoietin on platelet reactivity and thrombopoiesis in humans. *Blood* **2000**, *95*, 2983–2989.

(8) Yan, L.; Colandrea, V. J.; Hale, J. J. Prolyl hydroxylase domaincontaining protein inhibitors as stabilizers of hypoxia-inducible factor: small molecule-based therapeutics for anemia. *Expert Opin. Ther. Patents* **2010**, *20*, 1219–1245.

(9) Provenzano, R.; Fadda, G.; Bernardo, M.; James, C.; Kochendoerfer, G.; Lee, T.; Nakayama, S.; Neff, T.; Piper, B. A. FG2216, a novel oral HIF-PHI, stimulates erythropoiesis and increases hemoglobin concentration in patients with non-dialysis CK. *Am. J. Kidney Dis*. **2008**, *51*, B80.

(10) Roxadustat (FG-4592) met the primary endpoints in two Chinese Phase III trials to treat anemia in chronic kidney disease (CKD) patients, see: http://bciq.biocentury.com/products/asp1517.

(11) Maxwell, P. H.; Eckardt, K.-U. HIF prolyl hydroxylase inhibitors for the treatment of renal anaemia and beyond. *Nature Rev. Nephrology* **2016**, *12*, 157–168.

(12) Joharapurkar, A. A.; Pandya, V. B.; Patel, V. J.; Desai, R. C.; Jain, M. R. Prolyl Hydroxylase Inhibitors: A Breakthrough in the Therapy of Anemia Associated with Chronic Diseases. *J. Med. Chem.* **2018**, *61*, 6964-6982.

(13) Beck, H.; Jeske, M.; Thede, K.; Stoll, F.; Flamme, I.; Akbaba, M.; Ergeden, J.-K.; Karig, G.; Keldenich, J.; Oehme, F.; Militzer, H.-C.; Hartung, I. V.; Thuss, U. Discovery of Molidustat (BAY 85-3934): A Small-Molecule Oral HIF-Prolyl Hydroxylase (HIF-PH) Inhibitor for the Treatment of Renal Anemia, ChemMedChem **2018**, *13*, 988–1003.

(14) Vachal, P.; Miao, S.; Pierce, J. M.; Guiadeen, D.; Colandrea, V. J.; Wyvratt, M. J.; Salowe, S. P.; Sonatore, L. M.; Milligan, J. A.; Hajdu, R.; G., Anantha; K., C. A.; Lingham, R. B.; Mandala, S. M.; DeMartino, J. A.; Tong, X.; Wolff, M.; Steinhuebel, D.; Kieczykowski, G. R.; Fleitz, F. J.; Chapman, K.; Athanasopoulos, J.; Adam, G.; Akyuz, C. D.; Jena, D. K.; Lusen, J. W.; Meng, J.; Stein, B. D.; Xia, L.; Sherer, E. C.; Hale, J. J. 1,3,8-Triazaspiro[4.5]decane-2,4-diones as Efficacious Pan-Inhibitors of Hypoxia-Inducible Factor Prolyl Hydroxylase 1-3 (HIF PHD1-3) for the Treatment of Anemia. *J. Med. Chem*. **2012**, *55*, 2945–2959.

(15) Debenham, J. S.; Madsen-Duggan, C.; Clements, M. J.; Walsh, T. F.; Kuethe, J. T.; Reibarkh, M.; Salowe, S. P.; Sonatore, L. M.; Hajdu, R.; Milligan, J. A.; Visco, D. M.; Zhou, D.; Lingham, R. B.; Stickens, D.; DeMartino, J. A.; Tong, X.; Wolff, M.; Pang, J.; Miller, R. R.; Sherer, E. C.; Hale, J. J. Discovery of N-[Bis(4-methoxyphenyl)methyl]-4-hydroxy-2-(pyridazin-3-yl)pyrimidine-5-carboxamide (MK-8617), an Orally Active Pan-Inhibitor of Hypoxia-Inducible Factor Prolyl Hydroxylase 1-3 (HIF PHD1-3) for the Treatment of Anemia. *J. Med. Chem*. **2016**, *59*, 11039–11049.

(16) Semenza, G. L. Regulation of mammalian O_2 homeostasis by hypoxia-inducible factor 1. *Annu. Rev. Cell. Dev. Biol.* **1999**, *15*, 551–578.

(17) The HIF-PHD inhibitors, including FG-2216, that have reached phase 2 or 3 clinical trials did not show any toxicity related to pulmonary arterial hypertension thus far.

(18) Jelkmann, W. Erythropoietin: structure, control of production, and function. *Physiol. Rev*. **1992**, *72*, 449–489.

(19) Ball, M. K.; Waypa, G. B.; Mungai, P. T.; Nielsen, J. M.; Czech, L.; Dudley, V. J.; Beussink, L.; Dettman, R. W.; Berkelhamer, S. K.; Steinhorn, R. H.; Shah, S. J.; Schumacker, P. T. Regulation of hypoxia-induced pulmonary hypertension by vascular smooth muscle hypoxia-inducible factor-1α. *Am. J. Respir. Crit. Care Med.* **2014**, *189*, 314–324.

(20) Minamishima, Y. A.; Kaelin Jr., W. G. Reactivation of Hepatic EPO Synthesis in Mice After PHD Loss. *Science* **2010**, *329*, 407.

(21) Abrams, M. T.; Koser, M.; Burchard, J.; Strapps, W.; Mehmet, H.; Gindy, M.; Zaller, D.; Sepp-Lorenzino, L.; Stickens, D. A Single Dose of EGLN1 siRNA Yields Increased Erythropoiesis in Nonhuman Primates. *Nucl. Acid Ther*. **2014**, *24*, 405–412.

(22) See SI for experimental details.

(23) Smith, N. F.; Figg, W. D.; Sparreboom, A. Role of the liverspecific transporters OATP1B1 and OATP1B3 in governing drug elimination. *Expert Opin. Drug Metab. Toxicol*. **2005**, *1*, 429–445.

(24) Erion, M. D.; Bullough, D. A.; Lin, C.-C.; Hong, Z. HepDirect prodrugs for targeting nucleotide-based antiviral drugs to the liver. *Curr. Opin. Invest. Drugs* **2006**, *7*, 109–117.

(25) Kasuya, T.; Kuroda, S. Nanoparticles for human liver-specific drug and gene delivery systems: in vitro and in vivo advances. *Expert Opin. Drug Delivery* **2009**, *6*, 39–52.

(26) Compound **1** was from our internal systemic HIF-PHD program (unpublished results).

(27) Liu, X.; Testa, B.; Fahr, A. Lipophilicity and Its Relationship with Passive Drug Permeation. *Pharm. Res.* **2011**, *28*, 962–977.

(28) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *J. Med. Chem*. **2002**, *45*, 2615–2623.

(29) Also see: Cai, J.; Crespo, A.; Du, X.; Dubois, B. G.; Guiadeen, D.; Kothandaraman, S.; Liu, P.; Liu, R.; Quan, W.; Sinz, C.; Wang, L. Oxazinoquinolinecarboxamidoacetic acid derivatives as inhibitors of HIF prolyl hydroxylase and their preparation, WO 2016045127, **2016**.

(30) Dieckmann, W. *Ber. Dtsch. Chem. Ges.* **1894**, *27*, 102–103.

(31) For synthesis of **27**, also see: Cai, J.; Colandrea, V.; Crespo, A.; Debenham, J.; Du, X.; Guiadeen, D.; Liu, P.; Liu, R.; Madsen-Duggan, C. B.; McCoy, J. G.; Quan, W.; Sinz, C.; Wang, L. Quinolinecarboxamidoacetic acids and related compounds as inhibitors of HIF prolyl hydroxylase and their preparation, WO 2016045125, **2016**.

(32) For synthesis of **28**, also see: Cai, J.; Crespo, A.; Du, X.; Dubois, B. G.; Liu, P.; Liu, R.; Quan, W.; Sinz, C.; Wang, L. Tetrahydrofuropyridineecarboxamidoacetic acid derivatives as inhibitors of HIF prolyl hydroxylase and their preparation, WO 2016045128, **2016**.

(33) Pregnane X receptor (PXR) is a nuclear receptor known to regulate expression of drug-metabolizing enzymes including cytochrome P450 (CYP450), see: Chu, V., et al. In vitro and in vivo induction of cytochrome P450: A survey of the current practices and recommendations: A Pharmaceutical Research and Manufacturers of America perspective. *Drug Metab. Dispos*. **2009**, *37*, 1339–1354.