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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5616–5620

Design and synthesis of substituted pyridine derivatives as HIF-1α prolyl hydroxylase inhibitors

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Received 29 June 2006; revised 2 August 2006; accepted 2 August 2006

Abstract—Structure-guided de novo drug design led to the identification of a novel series of substituted pyridine derivatives as HIF-1α prolyl hydroxylase inhibitors. Pyridine carboxyamide derivatives bearing a substituted aryl group at the 5-position of the pyridine ring show appreciable activity, while constraining the side chain by placing a pyrazole carboxylic acid generated a potent lead series with consistent activity against EGLN-1.

Hypoxia inducible factor 1-alpha (HIF-1α) is a heterodimeric transcription factor that plays a key role in oxygen homeostasis in mammals, including humans.[1] Under hypoxic conditions (low partial pressure of oxygen), HIF-1 activates genes responsible for hypoxic response, such as angiogenesis (VEGF), erythropoiesis, and glycolysis.[2] Both heterodimer components, HIF-1α and HIF-1β (ARNT), are constitutively expressed and regulation is achieved by the selective destruction of HIF-1α. Therefore, HIF-1α plays a major role in cellular response to hypoxia.

HIF-1α is hydroxylated on prolines 564 and 402 by a family of non-heme, iron-containing prolyl hydroxylases (EGLN-1, EGLN-2, and EGLN-3) under normoxic conditions.[3–5] Upon hydroxylation, HIF-1α binds to the complex of proteins comprised of VHL (Von Hippel Lindau tumor suppressor protein), elongin C/B, CuI2, Rbx1, and ubiquitin ligase. This binding event targets HIF-1α for cytoplasmic proteasomal degradation. EGLN-mediated proline hydroxylation is strictly dependent on oxygen and consequently, under hypoxic conditions hydroxylation is inhibited—as a result, HIF-1α binds to HIF-1β to form a functional transcriptional activator which turns on genes with hypoxic response elements (e.g., VEGF, EPO, and glycolytic enzymes).[6] The upregulation of HIF activity has potential therapeutic significance for ischemic diseases including myocardial infarction, stroke, peripheral arterial disease, heart failure, diabetes, and anemia. We are interested in pharmacologically eliciting the hypoxic response by means of small-molecule prolyl hydroxylase inhibitors. Previously described HIF-1α prolyl hydroxylase inhibitors (compound **2**: approximate IC_{50} is 1 μM) have largely been 2-oxoglutarate (2-OG) mimetics (Fig. 1).[7]

Interestingly, the isoquinoline **3**, a recently disclosed HIF-1α prolyl hydroxylase inhibitor, consists of a non-peptidic-like structure and may inhibit EGLN enzymes by acting as a 2-OG mimetic (Fig. 2).[8]

In a significant breakthrough, we were recently able to solve the previously unknown crystal structure of EGLN-1 in complex with isoquinoline 3.[9,10]

The key interactions between compound **3** and the EGLN-1 active site are: the salt bridge between the carboxylic acid and Arg383, the coordination of iron via the isoquinoline nitrogen and the amide carbonyl, and

Keywords: Prolyl hydroxylase inhibitors; HIF-1α; Hypoxia; Ischemia; Peripheral arterial disease (PAD); Anemia.
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doi:10.1016/j.bmcl.2006.08.026

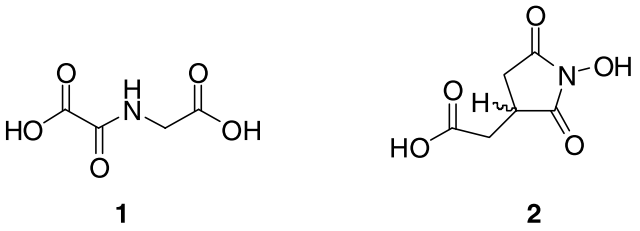


Figure 1. Small-molecule HIF-1α prolyl hydroxylase inhibitors.

the hydrogen bond between the phenolic hydroxyls of the compound and of Tyr303. While the in vitro activity of isoquinoline **3** on EGLN-1 assay was 1.4 μM, when either of the coordinating elements are absent (quinoline nitrogen or the amide carbonyl), those compounds exhibited no activity toward EGLN-1 enzyme in our hands. Thus, we decided to explore the metal coordinating ability of pyridine carboxyamides (see Fig. 3).

In our initial modeling studies, the substituted pyridine derivative **4** exhibited a reasonable binding orientation when docked into the EGLN-1 active site (Fig. 4).

In this binding orientation, the inhibitor carboxylate interacts with Arg383, while the pyridine nitrogen and the amide carbonyl coordinate iron. In addition, Met299 and the chloro-substituted benzene ring interact hydrophobically. It should be noted that the hydrogen bonding interaction that was observed with isoquinoline **3** is absent in this case, however, we observe an additional π-stacking interaction between the pyridine ring and Tyr310.

The substituted pyridine derivatives were synthesized according to the schemes below.

5-Bromopicolinic acid **5** was coupled with glycine methyl ester and a subsequent Suzuki coupling and a hydrolysis of the methyl ester generated the desired 5-aryl pyridine carboxyamides (Scheme 1). The 4-substituted pyridine carboxyamides were generated according to Scheme 2.

Next, we explored constraining the side chain with appropriate glycine isosteres to elicit more rigid interactions with the enzyme active site. The side-chain constrained pyridine analogs containing a pyrazole group

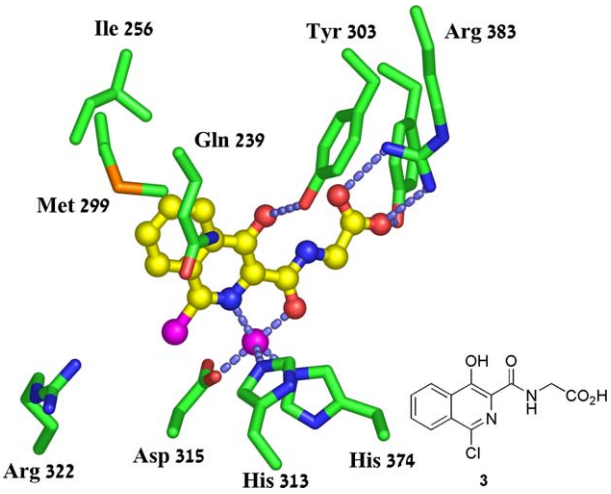


Figure 3. Binding of compound **3** to the active site of EGLN-1.

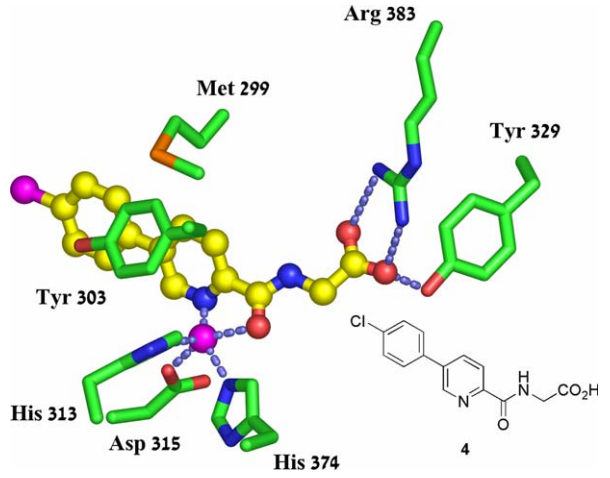


Figure 4. The possible binding mode of compound 4 to the active site of EGLN-1.

were synthesized via a microwave-assisted condensation reaction between 5-bromo-2-chloropyridine **11** and ethyl 1*H* pyrazolecarboxylate **12**. Suzuki coupling generated the desired analogs with concomitant hydrolysis of the ethyl ester under basic conditions (Scheme 3).

The substituted pyridine derivatives were screened against EGLN-1 enzyme assay (Table 1). The unsubstituted

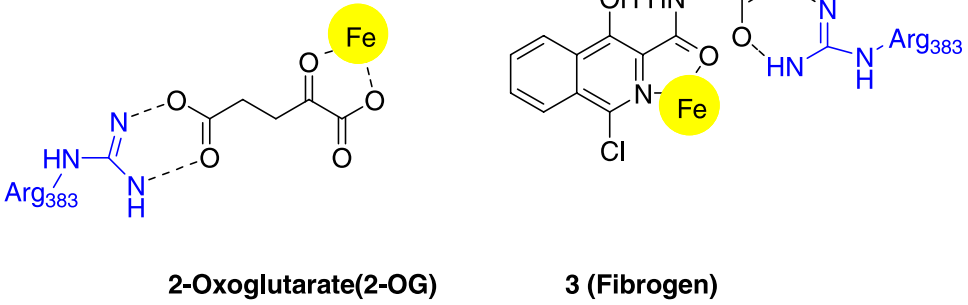
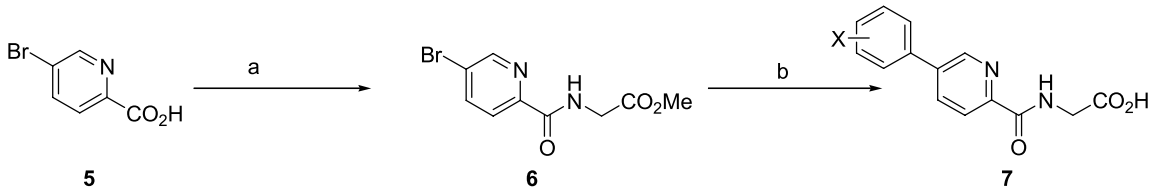
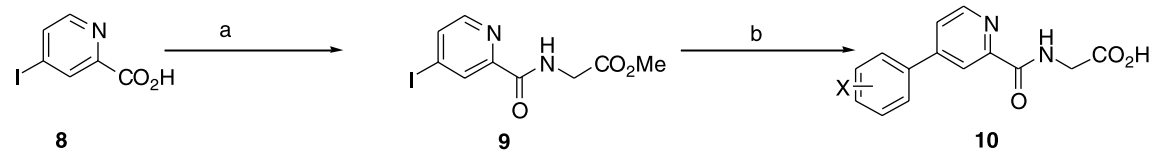


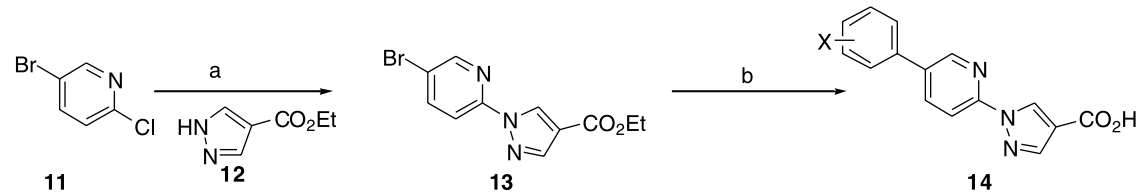
Figure 2. Possible binding modes of 2-OG and compound **3**.



Scheme 1. 5-Substituted pyridine derivatives. Reagents and conditions: (a) $SOCl_2$/Gly-OMe/iPr_2EtNH (90%); (b) 1—$ArB(OH)_2$/$Pd(dppf)Cl_2$/K_3PO_4/dioxane/EtOH; 2—LiOH/THF/Water (65%).



Scheme 2. 4-Substituted pyridine derivatives. Reagents and conditions: (a) EDAC/HOBt/Gly-OMe/iPr_2EtNH (52%); (b) 1—$ArB(OH)_2$/$Pd(dppf)Cl_2$/K_3P O_4/dioxane/EtOH; 2—LiOH/THF/water (65%).



Scheme 3. Reagents and conditions: (a) **12**/Cs_2CO_3/DMF/145 °C/MW/20 min (75%); (b) $ArB(OH)_2$/$Pd(PPh_3)_4$/K_2CO_3/CH_3CN/water/135 °C/MW/15 min (70%).

pyridine carboxyamide (Table 1, entry 1) was completely inactive. Similarly, the 4-substituted pyridine carboxyamide (Table 1, entry 2) did not show any activity. The 5-substituted pyridine carboxyamides showed discernable activity (Table 1, entries 3, 4, and 5). The *para*-chloro phenyl-substituted 5-pyridine carboxyamide (Table 1, entry 5) showed the best activity (15 μM). Interestingly, the *meta*-chloro derivative was completely inactive.

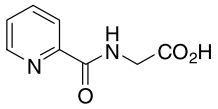
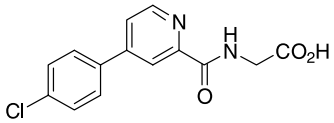
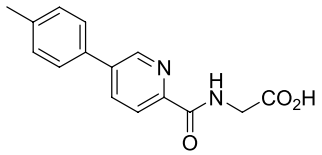
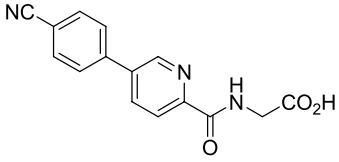
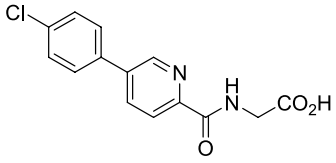
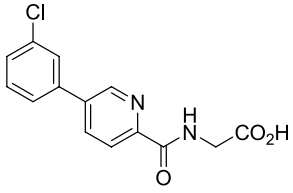
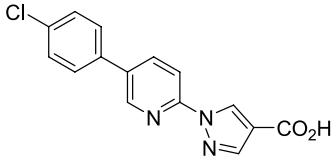
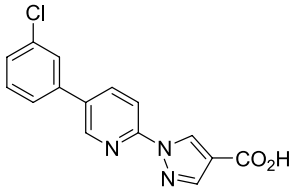
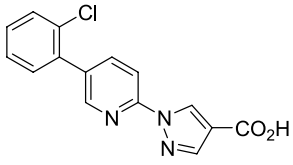
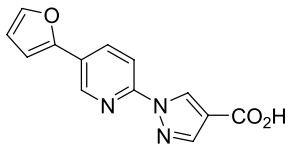
The side-chain-constrained, pyrazole substituted pyridine analogs exhibited consistently good activity. In this instance, chloro substitutions at the *ortho* and *meta* position at the outer benzene ring (Table 1, entries 8 and 9) were about three times more active than the *para*-chloro substituted compound (Table 1, entry 7). It was interesting to note that other heterocyclic glycine isosteres (e.g., oxazoles, imidazoles, and oxadiazole) exhibited weaker activity compared to the pyrazole isosteres.

In the case of electron-donation methoxy substitution the *para* methoxy substitution (Table 1, entry 13) was slightly more active than *ortho* and *meta* substitutions (Table 1, entries 15 and 16). Lipophilic substituents, for example, isopropyl (Table 1, entry 14) showed good activity while heteroaromatics, for example, thiophene (Table 1, entry 11), furan (Table 1, entry 10) and pyrimidine (Table 1, entry 12), showed a decrease in activity.

EGLN-1 activity assay. The EGLN-1 enzyme activity was determined using mass spectrometry (matrix-assisted laser desorption ionization, time-of-flight MS, MALDI-TOF MS). The HIF-1α peptide corresponding to residues 556–574 (DLDLEALAPYIPADDDFQL) was used as substrate. The reaction was conducted in a total volume of 50 μL containing Tris–Cl (5 mM, pH 7.5), ascorbate (120 μM), 2-oxoglutarate (3.2 μM), HIF-1α (8.6 μM), and bovine serum albumin (0.01%). EGLN-1, quantity predetermined to hydroxylate 20% of substrate in 20 min, was added to start the reaction. Where inhibitors were used, compounds were prepared in dimethylsulfoxide at 10-fold final assay concentration. After 20 min at room temperature, the reaction was stopped by transferring 10 μL of reaction mixture to 50μL of a mass spectrometry matrix solution (α-cyano-4-hydroxycinnamic acid, 5 mg/ml in 50% acetonitrile/0.1% TFA, 5 mM NH_4PO_4). Two microliters of the mixture was spotted onto a MALDI-TOF MS target plate for analysis with an Applied Biosystems (Foster City, CA) 4700 Proteomics Analyzer MALDI-TOF MS equipped with a Nd:YAG laser (355 nm, 3 ns pulse width, 200 Hz repetition rate). Hydroxylated peptide product were identified from substrate by the gain of 16 Da. Data defined as percent conversion of substrate to product was analyzed in GraphPad Prism 4 to calculate IC_{50} values.

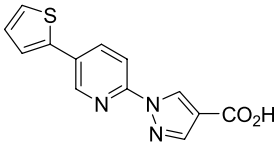
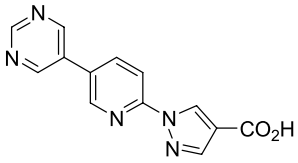
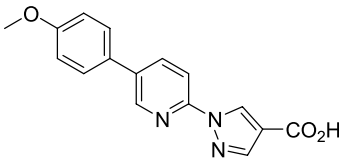
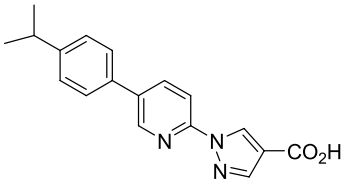
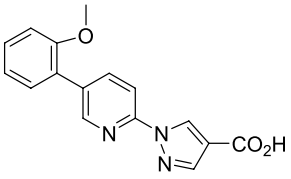
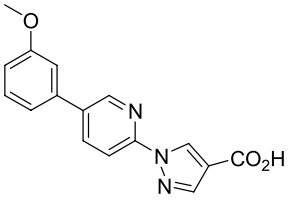
In conclusion, structure-based design approach and efficient chemistry strategy led us to discover a novel series of substituted pyridine derivatives as new HIF-1α prolyl hydroxylase inhibitors. This series served as initial lead compounds for a more potent compound series that were discovered by introducing

Table 1. Activity of substituted pyridine derivatives against EGLN-1 (95% CI)

Entry	Structure	EGLN-1 IC_{50} (μM)
1		>100
2		>100
3		46
4		39
5		15
6		>100
7		20
8		4.9
9		6.5
10		15

(*continued on next page*)

Table 1 (*continued*)

Entry	Structure	EGLN-1 IC_{50} (μM)
11		18
12		15
13		7.3
14		6.8
15		19
16		10

appropriate substitutions on the pyridine ring (details would follow). The EGLN-1 active compounds are currently under investigation for HIF-1 stabilization and cell-based VEGF induction (downstream biomarker for HIF-1 stabilization).

References and notes

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