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Giving Anemia a Boost with Inhibitors of Prolyl Hydroxylase

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ABSTRACT: There is much current interest in the development of inhibitors of the prolyl hydroxylase (PHD) enzymes that regulate the hypoxia-inducible transcription factor (HIF), which in turn stimulates the production of erythropoietin and ultimately red blood cells, as a treatment for anemia. A recent paper reports the synthesis and evaluation of a novel class of potent spirohydantoin-based pan-PHD inhibitors for this purpose. The paper is an exemplar of drug development from high-throughput screen to clinical candidate.

Anemia is a potentially serious consequence of several different diseases, with current treatments (primarily the administration of either red blood cells (RBCs) or recombinant erythropoietin) having considerable limitations of both cost and side effects. This has sparked recent efforts to develop alternative treatments, and Vachal et al.[1] discuss the development of a new class of spirohydantoin-based pan-inhibitors of prolyl hydroxylases (PHDs) as a potential treatment for this indication.

The prolyl hydroxylases (PHD1−3) are key regulators of the better-known heterodimeric, hypoxia-inducible transcription factor (HIF), which has primarily been of interest for its role in cancer but more recently also for its regulation of other key proteins, including erythropoietin.[2] Under normal oxic conditions HIF is maintained at very low levels by PHD hydroxylation (primarily by PHD2) of key prolyl residues on the essential HIFα subunit, tagging it for proteosomal degradation. Inhibition of PHDs, by preventing this process, has recently been considered as a way of increasing levels of HIF and thus erythropoietin, which itself has been clinically shown to stimulate the production of RBCs.[3] There has thus been considerable recent interest in PHD inhibitors for the treatment of anemia, with initial clinical trials reported for four compounds: FG-2216, FG-4592, GSK1278863A, and AKB-6548. The preliminary data reported from these trials are cautiously positive, although mechanisms are not yet clear and some toxicity issues are reported.[4]

Thus, it is exciting to see the paper by Vachal et al.,[1] who utilized an affinity selection mass spectrometry technique as an initial high-throughput screen, where PHD2 enzyme was incubated with mixtures of compounds and protein−ligand complexes were separated by size exclusion chromatography and identified by mass spectrometry. This resulted in a set of confirmed spiroindole hits of novel structure (e.g., **3h**, Figure 1) that were expanded by synthesis to give more potent compounds (e.g, **3ff**, PHD2 IC_{50} = 4 nM) but that had poor PK in rodent assays and unacceptable hERG activity. To address the PK liability, changes in the spiroindole core were studied, resulting in spirohydantoins such as **4f**, which had improved (although not optimal) PK properties and showed proof-of-concept in vivo, elevating erythropoietin levels in a mouse pharmacodynamic erythropoietin assay 4 h after drug delivery.

This was an interesting design move, since hydantoins have been tagged in the literature as "frequent hitters" or "PAINS",[5] structures that occur often as hits in high-throughput screens as promiscuous binders (and thus potentially with multiple biological activities and poor PK). Vachal et al. note the latter issue, which they suggest is a property of unsubstituted hydantoins, and avoided this by studying N,N-disubstituted analogues such as **6a**. The proof is that **6a** had a much better PK profile (C_{max} = 990 nM; $t_{1/2}$ = 1.1 h; AUC = 3.7 μM·h; F = 66%) than **4f** (C_{max} = 17 nM; $t_{1/2}$ = 0.12 h; AUC = 0.14 μM·h; F = 42%), despite having a free OH (although this is likely to be primarily in the pyrimidone form). Removal of the hERG liability could be achieved by a carboxylic acid functionality at any of several places on the molecule but preferably on the distal ring of the biphenyl moiety (e.g., **6q**). The final "off-target" issue was providing an adequate window between a therapeutic elevation in erythropoietin and an undesirable elevation in liver enzymes, exemplified by alanine transaminase. Since this balance was particularly affected by minor changes in the biphenyl moiety, an extensive structure−activity relationship study of this unit was carried out but did not provide a differential greater than that provided by **6q**. A final evaluation of further changes to the hydantoin N-heterocycle unit provided the clinical candidate **11l**, which showed a good PK profile (rat PK: C_{max} = 390 nM, $t_{1/2}$ = 1.1 h, F = 47%) and substantial elevation of erythropoietin levels (19 μg/L at a dose of 100 mg/kg iv) at 4 h, with no concomitant elevation in liver enzyme levels.

This report not only provides a new, potent, and orally bioavailable pan-PHD inhibitor of a new structural class to this therapeutic area but also provides a rare account of a complete drug development project, from initial high-throughput screening, identification of initial hits, improvement of enzyme, in vitro and in vivo potencies, and finally refinement of the toxicity profile and therapeutic window to produce a clinical candidate. The depth of effort involved is reflected by the large number of authors that made contributions to this very nice paper.

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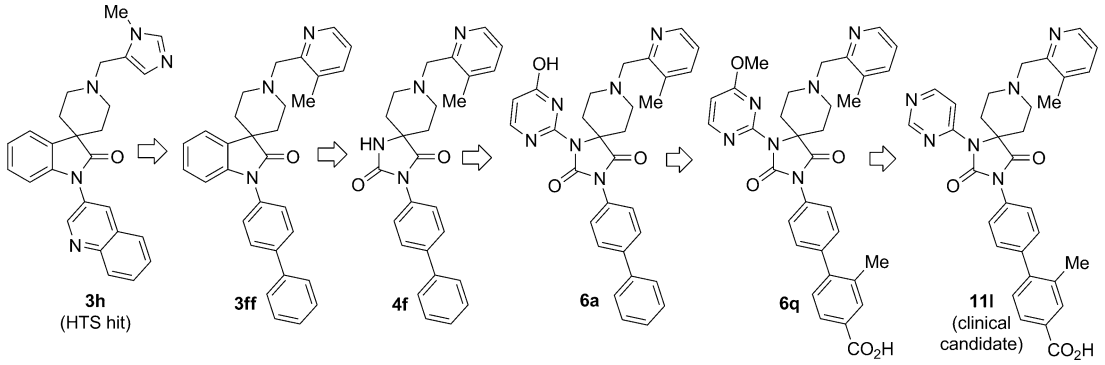

Figure 1. Design evolution from high-throughput screen hit **3h** to clinical candidate **11l**.

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REFERENCES

(1) 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.; Gollapudi, A.; Keohane, 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**, DOI: 10.1021/jm201542d.

(2) Jelkmann, W. Regulation of erythropoietin production. *J. Physiol.* **2011**, *589*, 1251−1258.

(3) Hand, C. C.; Brines, M. Promises and pitfalls in erythropoietin-mediated tissue protection: are non-erythropoietic derivatives a way forward? *J. Invest. Med.* **2011**, *59*, 1073−1082.

(4) Rabinowitz, M. H.; Barrett, T. D.; Rosen, M. D.; Venkatesan, H. Inhibitors of HIF prolyl hydroxylases. *Annu. Rep. Med. Chem.* **2010**, *45*, 123−139.

(5) Baell, J. B.; Holloway, G. A. New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem.* **2010**, *53*, 2719−2740.