Tetrahedron Letters 51 (2010) 952–954

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Parallel synthesis enablement of 2-pyridyl-5-cyano-pyrimidine-6-ones—anovel class of HIF-hydroxylase inhibitors

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ARTICLE INFO

Article history:
Received 17 November 2009
Revised 8 December 2009
Accepted 9 December 2009
Available online 16 December 2009

ABSTRACT

The synthesis of various substituted 2-pyridyl-5-cyano-pyrimidine-6-ones was accomplished via a three-step protocol. The key aspects of this parallel protocol was the preparation of amidines from nitriles under mild conditions followed by cyclization with ethyl (ethoxymethylene)cyanoacetate.

In response to hypoxia (a reduction in tissue oxygen level), a series of biological events are initiated and orchestrated by the transcription factor hypoxia inducible factor (HIF).[1] The response includes the generation of erythropoietin (EPO) stimulating erythropoiesis, production of VEGF inducing angiogenesis, as well as upregulation of genes involved in glucose uptake and energy metabolism.[2] Under normoxia conditions, HIF is hydroxylated on several proline residues by prolyl hydroxylase domain 1, 2, and 3 (PHD). This hydroxylation is required for von Hippel-Lindau tumor suppressor gene (VHL) recognition of HIF and subsequent degradation of HIF.[3] Recent data show that the inhibition of PHD-catalyzed hydroxylation blocks HIF degradation and induces HIF-mediated gene transcription, including the induction of EPO gene expression.[4] Thus we hypothesized that an HIF-hydroxylase inhibitor would be a potential therapy for anemia.[5]

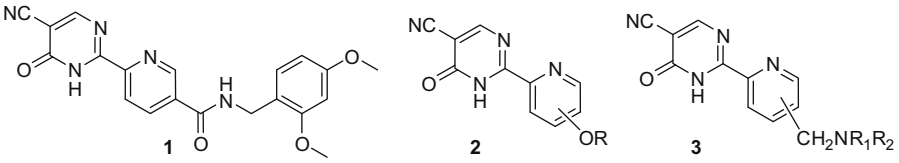
Compound **1** was identified by high throughput screening as a novel HIF-a prolyl hydroxylase inhibitor. The enzyme potency and in vitro and in vivo pharmacokinetic properties of **1** provided a promising starting point. However it displayed poor whole-cell activity, and solubility. To address these issues and also further optimize this hit, a robust method for rapid analog synthesis was needed. In particular, we were interested in developing protocols which would give ready access to ethers and amines such as **2** and **3**.

We hypothesized that construction of the cyano pyrimidone ring later in the synthesis as depicted in Scheme 1 would allow for easy access to the targets from readily available pyridol **4**[6] and the N-oxide **5**.

2-Cyano-5-hydroxy pyridine **4** was alkylated with commercially available primary and secondary alkyl bromides and chlorides utilizing polymer bound guanidine as the base.[7] The greater diversity afforded by commercial and internal alcohols (**6**) was used to our advantage by in situ methane sulfonate activation followed by alkylation using Cs_2CO_3 as base to afford the desired pyridyl ethers **7** in modest yields (Scheme 2).

The next step was conversion of the cyano group to the corresponding amidine. While a number of methods are available to execute this transformation most of them used strongly acidic conditions and/or involved the use of highly moisture-sensitive reagents or unstable intermediates (Scheme 3).[8]

To avoid such conditions, we developed a more robust amidine synthesis that can be used in a parallel protocol with a diverse set of starting nitriles by the use of *N*-acetyl cysteine and ammonia (Scheme 4).[9] While this procedure has been used to prepare a number of thrombin inhibitors on scale[10] use of the method for parallel synthesis of amidines is unreported. Initially we found that heating a mixture of the nitriles **7** with 1 equiv of *N*-acetyl cysteine



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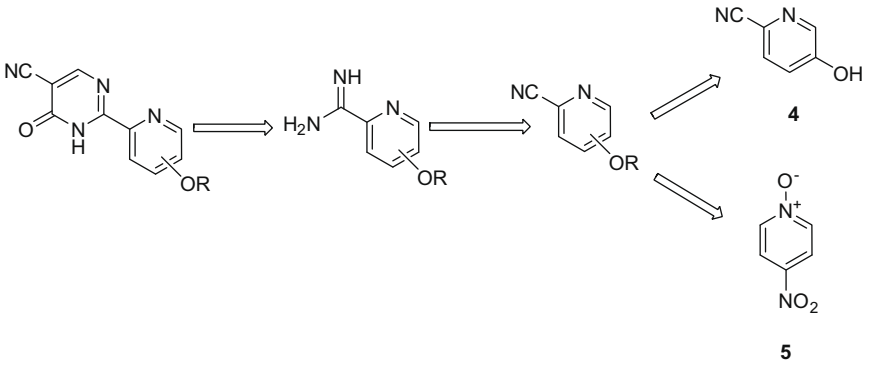
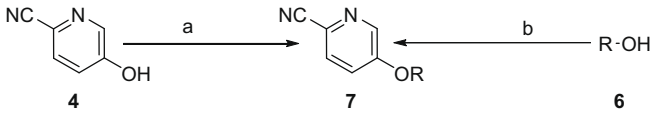
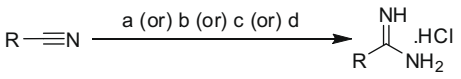
doi:10.1016/j.tetlet.2009.12.040



Scheme 1. Retrosynthesis of targets.



Scheme 2. Reagents and conditions: (a) RBr or RCl, P-TBD, CH_3CN, 70 °C, 60–70%; (b) (i) CH_3SO_2Cl, DIEA, DCE; (ii) **4**, Cs_2CO_3, DMF, 70 °C, 50–60%.



Scheme 3. Reagents and conditions: (a) (i) EtOH, HCl(g), (ii) NH_3(g), EtOH; Ref. 8a; (b) (i) NaOMe, MeOH; (ii) NH_4Cl, MeOH; Ref. 8b; (c) $MeAl(Cl)NH_2$, toluene, 80 °C, Ref. 8c; (d) (i) NaHMDS; (ii) HCl; Ref. 9.

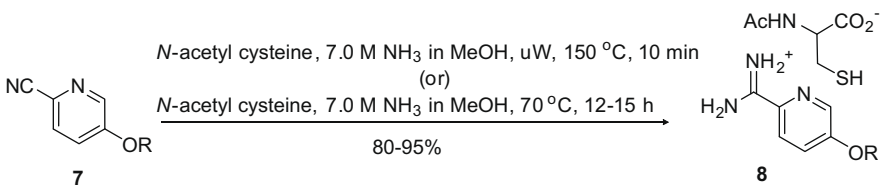
in 7.0 M NH_3 in methanol at 150 °C for 10 min under microwave irradiation yielded quantitative conversion to the amidines **8** as their *N*-acetyl cysteine salts. More importantly, this method allows for easy isolation of the amidine by concentration of the reaction mixture. Although the synthesis of amidines was facile under microwave conditions, to completely convert the nitriles to amidines necessitated reaction concentration of 0.7 M or higher. Due to the limitation of the vial size available for commercial microwave instruments, the reactions required a minimum 0.5 mmol of substrate to successfully run amidine formation at the desired 0.7 M concentration. Since most parallel chemistry operations are usually done with 0.1–0.25 mmol of substrate, we looked for an alternate mode of heating which would allow for rapid generation of amidines in parallel. After considerable experimentation, we found that heating a 0.7 M solution of an equimolar mixture of nitrile substrate and *N*-acetyl cysteine in 7.0 M NH_3 in a sealed vial at 70 °C for 12–15 h provided near quantitative yields of the desired amidines as their *N*-acetyl cysteine salt.

With a parallel method for preparation of desired amidines in hand, we turned our attention to their conversion to the target pyrimidines **9**. Reaction of the amidines with ethyl (ethoxymethylene)cyanoacetate in ethanol in the presence of sodium ethoxide afforded a mixture of the amino ester **10** and the desired pyrimidone **9** in ratios ranging from 1:1 to 1:4.[11] Although difficult to separate, we found that the undesired amino ester was easily removed from the reaction mixture by a solid phase extraction using commercial strong cation exchange (MCX) resin.[12] This catch and release method proved to be superior to simple acid base extractive separation due to the weakly basic nature of the by-product (**10**) (Scheme 5). This methodology was successfully used to prepare an 80-membered library of **9** in overall yields ranging from 20% to 30% starting from compound **4**. A number of potent HIF-hydroxylase inhibitors with improved whole-cell activity were identified from this library.

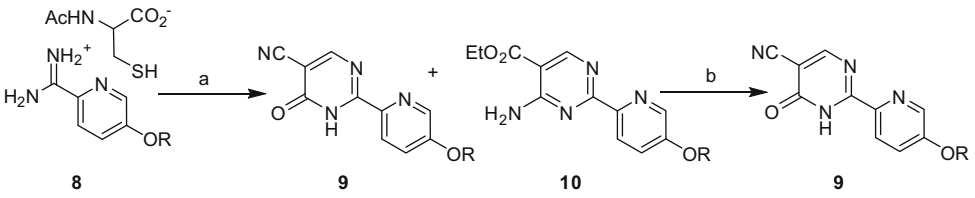
A similar alkylation approach to 4-substituted ethers was developed via 4-nitro-pyridine-N-oxide (**5**). Treatment of **5** with alkoxides[13] followed by reaction with TMSCN[14] gave the desired 4-alkoxy-2-cyano pyridines **11** in modest yield. This material was carried through a similar sequence as that of the 3-alkoxy derivatives to provide the desired targets **12** (Scheme 6).

We next turned our attention to the preparation of benzylamine analogs **3**. These amines were accessed via pyrimidone aldehyde derivatives **15** and subsequent reductive amination with amines (Scheme 7). Since diversity was introduced in the last step, we decided to prepare aldehyde templates **15** on larger scale using established procedures.[8b] Thus, treatment of cyano ketals **13** with catalytic sodium ethoxide in ethanol and solid ammonium chloride provided intermediate amidines. Without isolation, these amidines were treated with additional sodium ethoxide and ethyl (ethoxymethylene)cyanoacetate to directly access ketals **14** in modest yields. Unmasking of the aldehyde was accomplished in neat formic acid thus avoiding extractive isolation.

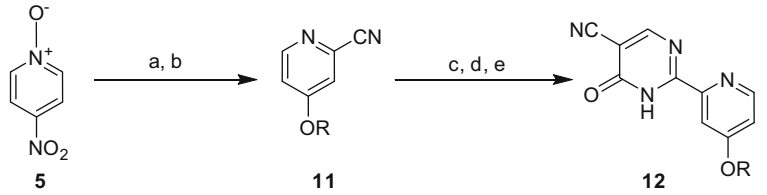
In summary, parallel chemistry protocols were developed for rapid synthesis and exploration of analogs related to HIF-hydroxylase inhibitor **1**.[15] More than 250 analogs were prepared in library format using the methodology described here. Key aspects of the approach described here are: an operationally simple parallel synthesis of amidines from corresponding nitriles and use of catch and release in the purification of by product formed during condensation of the amidines with 1,3-diketone derivatives. Derivatives from this approach were more potent than **1** and increased EPO



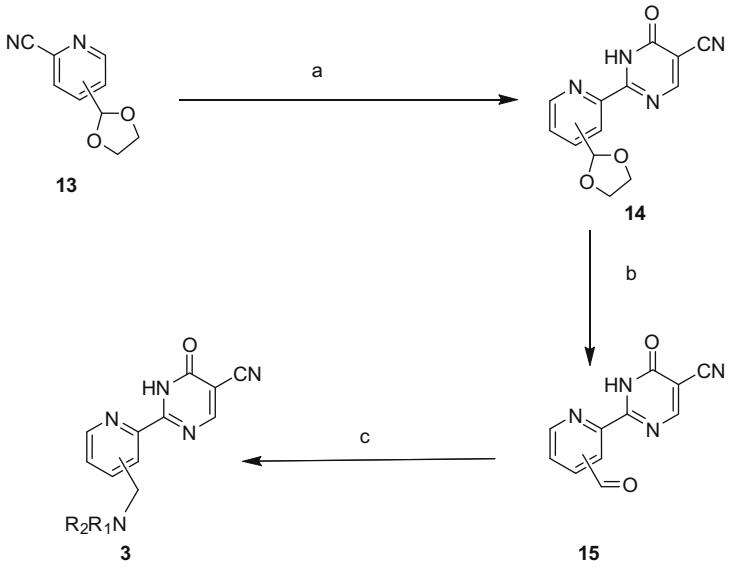
Scheme 4. Synthesis of amidines.



Scheme 5. Reagents and conditions: (a) ethyl (ethoxymethylene)cyanoacetate, NaOEt, EtOH; (b) (i) MCX catch and release; (ii) reverse phase HPLC; overall 50–60% from **8**.



Scheme 6. Reagents and conditions: (a) ROH, NaH, rt, 80%; (b) TMSCN, *N*,*N*-diethylcarbamoyl chloride, DCM, rt, 12 h, 90%; (c) *N*-acetyl cysteine, 7.0 M NH_3 in MeOH, μW, 150 °C, 5 min (or) *N*-acetyl cysteine, 7.0 M NH_3 in MeOH, 70 °C, 12–15 h; (d) ethyl (ethoxymethylene)cyanoacetate, NaOEt, ethanol; (e) MCX followed by reverse phase purification: 25–30% overall yield from **5**.



Scheme 7. Reagents and conditions: (a) (i) EtOH/NaOEt; (ii) NH_4Cl; (iii) ethyl (ethoxymethylene)cyanoacetate, EtOH, NaOEt, 60 °C, 60%; (b) 98% formic acid, 50 °C, 95%; (c) R_1R_2NH, MP-$CNBH_3$, THF, MeOH, HOAc, rt, 50–60%.

levels in mice when administered orally. Detailed biological SAR from this approach will be the subject of future publications.

Acknowledgments

The authors would like to thank Christopher J. O'Donnell, Jotham Coe, Andy Flick, and Martin Petterson for carefully reading early drafts of this manuscript and providing useful suggestions to improve it.

References and notes

1. (a) Semenza, G. L. *Biochem. J.* **2007**, *405*, 1; (b) Stockmann, C.; Fandrey, J. *Clin. Exp. Pharmacol. Physiol.* **2006**, *33*, 968.
2. Semenza, G. L. *Physiology* **2004**, *19*, 176.
3. For a recent review on HIF biology, see: Smith, T. G.; Robbins, P. A.; Ratcliffe, P. J. *Br. J. Haematol.* **2008**, *141*, 325.
4. (a) Rankin, E. B.; Biju, M. P.; Liu, Q.; Unger, T. L.; Rha, J.; Johnson, R. S.; Celeste, S. M.; Keith, B.; Haase, V. H. *J. Clin. Invest.* **2007**, *117*, 1068; (b) Ratcliffe, P. J. *J. Clin. Invest.* **2007**, *117*, 862.
5. (a) Semenza, G. L. *Expert Opin. Ther. Targets* **2006**, *10*, 267; (b) Hsieh, M. M.; Linde, N. S.; Wynter, A.; Metzger, M.; Wong, C.; Langsetmo, I.; Lin, A. L.; Smith, R.; Rodgers, G. P.; Donahue, R. E.; Klaus, S. J.; Tisdale, J. F. *Blood* **2007**, *110*, 2140; (c) FG-2216, an orally active HIF-a-inhibitor has been reported to be effective in increasing EPO levels in the clinic *Drug News and Perspectives* **2007**, *20*, 647.
6. Wong, N. C. W.; Tucker, J. E. L.; Hansen, H. C.; Chiacchia, F. S.; McCaffrey, David. WO 2006045096, 2006.
7. Xu, W.; Mohan, R.; Morrissey, M. M. *Tetrahedron Lett.* **1997**, *38*, 7337.
8. (a) Pinner, A. *Ber* **1885**, *18*, 2845; (b) Schaefer, F. C.; Peters, G. A. *J. Org. Chem.* **1961**, *26*, 412; (c) Garigipati, R. S. *Tetrahedron Lett.* **1990**, *31*, 1969; (d) Boere, T. R.; Oakley, R. T.; Reed, R. W. *J. Organomet. Chem.* **1987**, *331*, 161.
9. Lange, U. E. W.; Schafer, B.; Baucke, D.; Buschmann, E.; Mack, H. *Tetrahedron Lett.* **1999**, *40*, 7067.
10. Bernard, H.; Buelow, G.; Lange, U. E. W.; Mack, H.; Pfeiffer, T.; Schaefer, B.; Seitz, W.; Zierke, T. *Synthesis* **2004**, *14*, 2367.
11. Lorente, A.; Vaquerizo, L.; Martin, A.; Gomez-Sal, P. *Heterocycles* **1995**, *41*, 71.
12. MCX cartridges for catch and release were purchased from Waters corporation.
13. Ochiai, E. *J. Org. Chem.* **1953**, *18*, 534.
14. Fife, W. K. *J. Org. Chem.* **1983**, *48*, 137.
15. All new compounds were characterized by full spectroscopic data: ^1H NMR and LC/MS for singletons and by LC/MS for libraries. Yields refer to chromatographed material with purity >95% for singletons and >85% for library analogs.