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Synthesis and evaluation of quinazolin-4-ones as hypoxia-inducible factor-1α inhibitors

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ABSTRACT

Quinazolin-4-one **1** was identified as an inhibitor of the HIF-1α transcriptional factor from a high-throughput screen. HIF-1α up-regulation is common in many cancer cells. In this Letter, we describe an efficient one-pot sequential reaction for the synthesis of quinazolin-4-one **1** analogues. The structure–activity relationship (SAR) study led to the 5-fold more potent analogue, **16.**

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Hypoxia-inducible factor (HIF-1) is a dimeric transcription factor consisting of an oxygen regulated α-component and a constitutively expressed β-component. At normal oxygen levels, HIF-1α is degraded via the pVHL-mediated ubiquitin-proteosomal pathway. Under hypoxic conditions, HIF-1α rapidly accumulates and dimerizes with HIF-1β. This heterodimer binds to the DNA hypoxia-response element (HRE) and activates a diverse array of target genes.[1] This pathway is particularly relevant to the cancer field because oxygen levels in tumors are commonly lower than in the surrounding tissues. Hypoxic cells are resistant to radiation damage and their distances from blood vessels reduce the potency of anti-cancer drugs. Hypoxia additionally promotes the up-regulation of genes involved in drug resistance. HIF-1 is directly responsible for the induction of numerous genes that are present at higher levels in cancer cells, in particular VEGF. The overexpression of HIF-1 has been related to the aggressiveness and vascularity of tumors, and mortality rate in patients. Despite the introduced difficulties in treating hypoxic tumors, the hypoxic environment found in tumor cells can be exploited for targeted therapy. One strategy to achieve this involves the identification of HIF-1 inhibitors as potential anti-cancer drugs.[2] We recently reported a high-throughput cell-based HIF-1 mediated β-lactamase reporter gene assay. Upon screening a library of 73,000 compounds (PubChem AID:915 (http://pubchem.ncbi.nlm.nih.gov)), several compounds were identified as novel inhibitors of the HIF-1 signaling pathway.[3] Among these hits, quinazolin-4-one **1** (NCGC00056044) showed good drug-like properties and was selected for further exploration.

Three areas were selected for structure-activity relationship (SAR) studies: (1) substitution in area A; (2) piperazine region B; and (3) phenyl substitution in area C (Fig. 1).

To facilitate our compound synthesis for the SAR study, we modified a reported method[4] to remove the need for intermediate purification. In addition, a microwave reactor was used to accelerate the synthesis. Acylation of anthranilic acid **2** with chloroacetyl chloride gave **3,** which was treated with aniline **4** to afford chloride **5** (Scheme 1). The chloride was reacted with amine **6** to give compounds **1**, **7–36**. All three steps were conducted in one-pot without

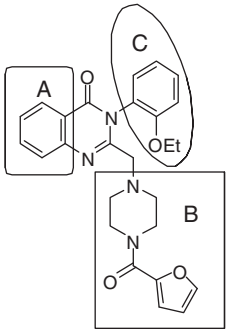

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Figure 1. Optimization plan for NCGC00056044 (**1**).

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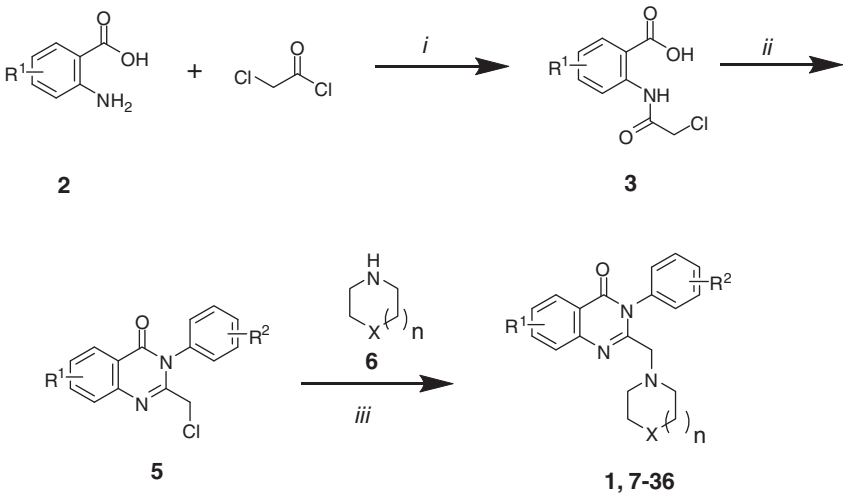
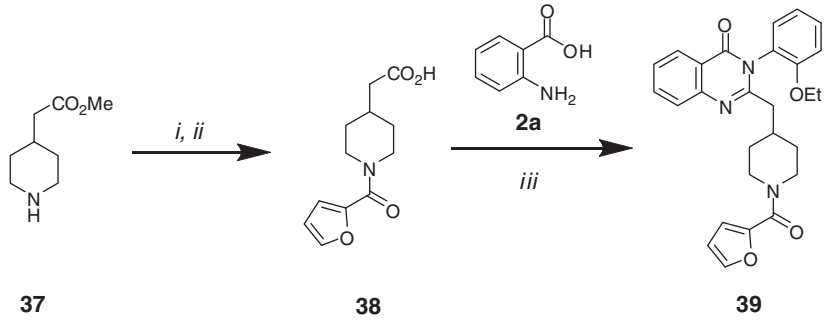


Scheme 1. Reagents and conditions: (i) *i*PrNEt$_2$, ACN, rt; (ii) ArNH$_2$ (**4**), POCl$_3$, MW 150 °C, 15 min; (iii) K$_2$CO$_3$, EtOH, MW 150 °C, 5 min; then amine **6**, MW 150 °C, 10 min.



Scheme 2. Reagents and conditions: (i) *i*PrNEt$_2$, DCM, 2-furoyl chloride; (ii) LiOH; (iii) 2-ethoxyaniline, **2a**, pyridine, MW 230 °C, 10 min.

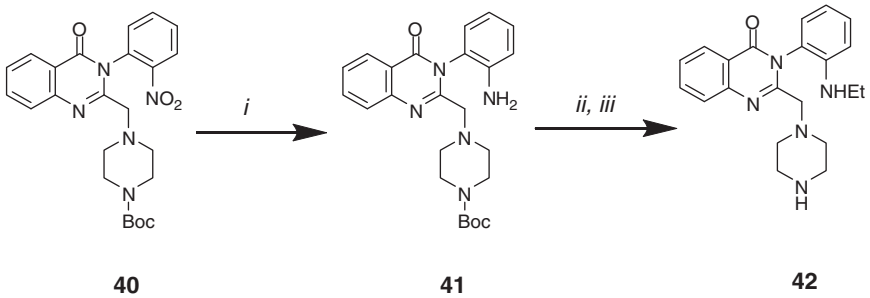
the need for intermediate isolation. This protocol was carried out in a parallel fashion to prepare the analogues which were purified via HPLC.[5]

Compound **39** was prepared as described in Scheme 2. Reaction of **37** with 2-furoyl chloride, followed by a hydrolysis reaction yielded acid **38**. The desired **39** was obtained via a microwave assisted one-pot three-component reaction of **38**, acid **2a**, and 2-ethoxyaniline.[6]

Scheme 3 describes the synthesis of the area C analogue **42.** Nitro-reduction of **40** gave **41**. Alkylation of the aniline nitrogen in **41** using ethyl iodide followed by a Boc-deprotection gave **42**.

All analogues were evaluated in a cell-based HIF-1 mediated β-lactamase reporter gene assay under hypoxic conditions.[7] Area A showed little tolerance for substitution (Table 1). The C-6 methoxy (**7**), C-5 iodo (**9**), and C-4 and C-5 dimethoxy (**10**) substitutions were inactive. Compound **8** with a methyl group at C-6 was active, but it was 3-fold less potent than the original hit (**1**). Considering these results, our efforts focused on the optimization of areas B and C (Fig. 1).

Modification of the piperazine region B is shown in Table 2. Acetylation of N-4 (**11**) resulted in similar activity to the hit compound (**1**), but capping the piperazine nitrogen with a benzamide (**12**) or ethyl carbamate (**13**) resulted in a loss of activity. N-4 methylation (**14**) or benzylation (**15**) resulted in a 2-fold and 64-fold loss of activity respectively. Ultimately, the most active compound was the unsubstituted N-4 analogue (**16**), which was about 5-fold more potent than **1**. N-4 was critical for activity because when it was replaced with either a carbon (**19**) or oxygen (**18**), activity was lost.



Scheme 3. Reagents and conditions: (i) $Na_2S_2O_4$; MW 100 °C, 10 min (ii) EtI, DMF, *i*Pr$_2$NEt, K$_2$CO$_3$, MW 150 °C, 15 min; (iii) DCM, TFA, rt 2 h.