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Synthesis and structure–activity relationships of novel, potent, orally active hypoxia-inducible factor-1 inhibitors

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ABSTRACT

Hypoxia-inducible factor-1 (HIF-1) is the chief transcription factor regulating hypoxia-driven gene expression. HIF-1 overexpression is associated with poor prognosis in several cancers and therefore represents an attractive target for novel antitumor agents. We explored small molecule inhibitors of the HIF-1 pathway. Using high-throughput-screening, we identified benzanilide compound **1** (IC_{50} = 560 nM) as a seed. Subsequent extensive derivatization led to the discovery of compounds **43a** and **51d**, with anti-HIF-1 activities in vitro (IC_{50} = 21 and 0.47 nM, respectively), and in vivo. Additionally, **43a** (12.5–100 mg/kg) also displayed in vivo anti-tumor efficacy, without influencing body weight.

1. Introduction

It is generally accepted that cancer cells in hypoxic regions resist existing chemotherapies and radiation therapy,[1–3] and are associated with an increased risk of invasion, metastasis, treatment failure, and patient mortality.[3–5] Hypoxia in itself produces various disadvantages for both normal and cancer cells, such as decreased energy production and induction of apoptosis. However, cancer cells can adapt to the hypoxic environment by activating the hypoxia-inducible factor (HIF) pathway to induce the expression of a wide variety of genes involved in glycolysis, angiogenesis, hematopoiesis, survival pathways, and invasion.[6–10] HIF-1 is a transcription factor, which consists of an α/β heterodimer. HIF-1α is an oxygen-regulated protein and HIF-1β is a constitutively expressed protein, irrespective of oxygen concentration. Under normoxic conditions, the von Hippel–Lindau (VHL) protein recognizes and binds to HIF-1α, which is hydroxylated by prolyl hydroxylase-domain proteins (PHD) 1–3 on proline residues 402 and/or 564. Subsequently, HIF-1α is polyubiquitinated and rapidly degraded by the 26S proteasome. Under hypoxic conditions, however, HIF-1α is highly stabilized, dimerizes with HIF-1β, and translocates to the nucleus. The HIF-1 complex then becomes transcriptionally activated and contributes to cancer malignancy.[6–12]

Overexpression of HIF-1α has been reported in many types of cancers, including colon, breast, lung, gastric, skin, ovarian, pancreatic, prostate and renal carcinomas.[13] It is significantly associated with patient mortality in several different types of cancers affecting the brain, breast, cervix, and lung.[5] These findings indicated that HIF-1α represented an attractive therapeutic target for a wide range of cancers and considerable effort has therefore been devoted to the identification of HIF-1 inhibitors. Various types of small molecules have been reported to inhibit the HIF-1 pathway.[14–16] However, no HIF-1 pathway inhibitors have yet been approved for clinical use and there is still a need for a novel compound with potent HIF-1 inhibitory activity.

The present study describes our identification of novel HIF-1 inhibitors. High-throughput screening (HTS) using a cell-based reporter assay[17] identified *N*-(4-iodophenyl)-4-(morpholinomethyl)-benzamide (compound **1**), which had submicromolar activity (IC_{50} = 560 nM) under hypoxic conditions. This compound reduced

Abbreviations: HIF-1, hypoxia-inducible factor-1; VHL, von Hippel–Lindau; PHD, prolyl hydroxylase; HTS, high-throughput screening; NBS, *N*-bromosuccinimide; MNBA, 2-methyl-6-nitrobenzoic anhydride; DIBAL, diisobutylaluminum hydride; VEGF-PLAP, vascular endothelial growth factor-placental alkaline phosphatase.

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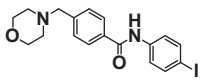
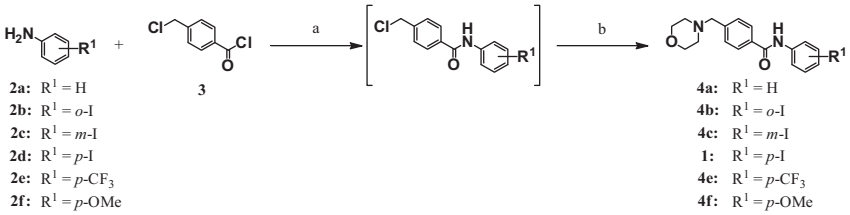


Figure 1. Benzanilide compound **1**.

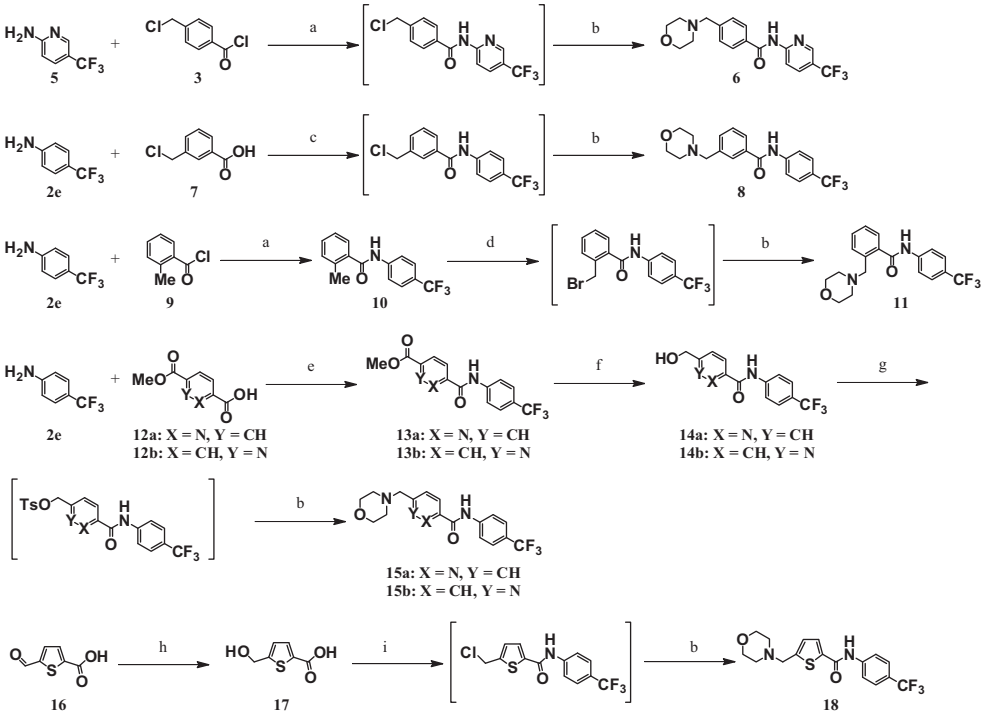
the HIF-1α protein level without affecting its mRNA level.[18] Based on these data, compound **1** was derivatized to produce compounds that showed in vitro activity at subnanomolar concentrations and in vivo efficacy in the U251 mouse xenograft model, without changes in body weight.

2. Chemistry

First, we explored the optimal substitution of the *para*-iodo moiety of benzanilide compound **1** (Fig. 1) to create more potent compounds. Derivatization of benzanilide was conducted as shown in Scheme 1. Benzanilide derivatives, **1** and **4a–c**,**e**,**f**, were synthesized by condensation followed amidation. To investigate the optimal position of a substituent, *ortho*-, *meta*-, and *para*-benzylmorpholine were prepared for the amide structure using similar synthetic routes (Scheme 2).[19] To explore an alternative to the benzanilide structure, we synthesized compounds bearing a heteroaromatic core. Condensation was carried out either using 2-methyl-6-nitrobenzoic anhydride (MNBA) or via acid chloride from **12**, followed by reduction using lithium borohydride. Subsequent tosylation and amination with morpholine gave **6**, **15a–b** with pyridine, and **18** with thiophene. These benzanilide compounds had some sub-optimal physicochemical properties, as they were highly crystalline, and/or raised concerns regarding their chemical/metabolic amide stability. With the aim of improving these physicochemical properties, we explored isosteres of the amide moiety or alternatives to morpholine. *E*-Olefine **21** was synthesized from phosphonate **19**[20] and aldehyde **20** using a conventional Horner–Wadsworth–Emmons reaction.[21] The subsequent reduction of methyl ester, oxidation of alcohol and reductive amination afforded the desired *E*-olefine compound **24** (Scheme 3).[22–24] Compounds **26**, **27**, and **30** were synthesized from the already known compounds **25**, **1**, and **28**, respectively. Compounds **32a–d** and **35a–d** were synthesized via nucleophilic substitution to benzylchloride **31** using the amine derivative (Scheme 4).[25] Compound **38** without benzylamine was formed using **2d** and **36**. Sonogashira cross-coupling reactions with aromatic or cyclic aliphatic acetylene using Pd and CuI as catalysts produced the corresponding compounds **41a–e** and **43a–b**, respectively (Scheme 5).[26–28] Compounds **51a**,**c**,**d** were prepared from



Scheme 1. Reagents and conditions: (a) saturated $NaHCO_3$ aq, THF, room temperature (RT) or Et_3N, THF, RT; (b) morpholine, RT or morpholine, THF, RT.



Scheme 2. Reagents and conditions: (a) saturated $NaHCO_3$ aq, THF, room temperature (RT) or Et_3N, THF, 0 °C; (b) then morpholine, RT, or morpholine, Et_3N, DMF, RT; (c) $SOCl_2$, reflux then saturated $NaHCO_3$ aq, THF, RT; (d) NBS, benzoyl peroxide, CCl_4, reflux; (e) $SOCl_2$, reflux then Et_3N, THF, RT or MNBA, Et_3N, DMAP, DCM, RT; (f) $LiBH_4$, THF, RT; (g) TsCl, DMAP, pyridine, Et_3N, THF–DCM, RT; (h) $NaBH_4$, MeOH, 0 °C to RT; (i) $SOCl_2$, reflux then H_2N–C_6H_4–CF_3, Et_3N, THF, RT.