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Design, synthesis and biological activity of 3-pyrazine-2-yl-oxazolidin-2-ones as novel, potent and selective inhibitors of mutant isocitrate dehydrogenase 1

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ABSTRACT

Isocitrate dehydrogenases (IDHs) catalyze the oxidative decarboxylation of isocitrate to alpha-ketoglutarate (α-KG) generating carbon dioxide and NADPH/NADH. Evidence suggests that the specific mutations in IDH1 are critical to the growth and reproduction of some tumor cells such as gliomas and acute myeloid leukemia, emerging as an attractive antitumor target. In order to discovery potent new mutant IDH1 inhibitors, we designed, synthesized and evaluated a series of allosteric mIDH1 inhibitors harboring the scaffold of 3-pyrazine-2-yl-oxazolidin-2-ones. All tested compounds effectively suppress the D-2-hydroxyglutarate (D-2-HG) production in cells transfected with IDH1-R132H and IDH1-R132C mutations at 10 μM and 50 μM. Importantly, compound **3g** owns the similar inhibitory activity to the positive agent **NI-1** and shows no significant toxicity at the two concentrations. The parallel artificial membrane permeation assay of the blood–brain barrier (PAMPA-BBB) identified **3g** with a good ability to penetrate the blood–brain barrier (BBB). These findings indicate that **3g** deserves further optimization as a lead compound for the treatment of patients with IDH1 mutated brain cancers.

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1. Introduction

Isocitrate dehydrogenases (IDHs), a key enzyme family associated with the tricarboxylic acid (TCA), catalyze oxidative decarboxylation of isocitrate acid to α-ketoglutaric (α-KG) using divalent magnesium ion and $NADP^+$ (or NAD^+) as cofactors, which is critical to biosynthesis and energy metabolism.[1] Isocitrate dehydrogenase 1 (IDH1), one of the IDHs, locates in the cytosol and peroxisome, while IDH2 and IDH3 in mitochondria. IDH1 is the only NADPH producer in the biosynthesis and the thiolated antioxidant system and plays a vital role in the process of adipogenesis and phospholipid metabolism.[2]

Recent studies revealed that IDH1 is a metabolic enzyme associated with the progression of cancers, and mutant IDH1 (mIDH1) primarily exists in gliomas, acute myeloid leukemia and other solid tumors.[3,4] Substantial data suggests that more than 70% low-grade gliomas and up to 20% secondary glioblastoma multiform are related to IDH1 mutations.[5] In addition, mutant IDH1 is also found in about 10% of AML cases and 10% of cholangiocarcinomas, as well as melanomas and chondrosarcomas.[5] The most common mutation in IDH1 is a key amino acid residue Arg132 located in the active site, in which R132H is the predominant.[6]

Mutations in IDH1 are heterozygous missense mutations which lead to an arginine at amino acid 132 replaced by different amino

Abbreviations: IDH, Isocitrate dehydrogenase; TCA, the tricarboxylic acid; a-KG, a-Ketoglutaric acid; D2HG, D-2-Hydroxyglutaric acid; mIDH1, Mutant IDH1; mIDH2, Mutant IDH2; R132H, Arg132 mutation to His; R132C, Arg132 mutation to Cys; R140Q, Arg140 mutation to Gln; WT, wild type; BBB, Blood Brain Barrier.

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acid residues, including R132H, R132C, R132L, R132G, R132S and R132W.[2] mIDH1 exhibits a neomorphic catalytic activity to catalyze the conversion of α-KG to D-2-hydroxyglutarate (D-2-HG).[7] Comparably, IDH1 mutated cells show higher concentrations of D-2-HG than normal cells, which can be as a significant evaluation of certain tumors. D-2-HG, an oncometabolite related to tumorigenesis, induces hypermethylation of histone and chromatin and blocks cell differentiation through competitive inhibition with relevant α-KG-dependent dioxygenases, such as methylases and histone demethylases.[8,9] Therefore, high level of D-2-HG from IDH1 mutations are sufficient to promote the initiation and progression of cancers, such as gliomas and acute myeloid leukemia.[10] Taken these findings into consideration, it is demonstrated that potent drugs targeting mutant IDH1 should be developed for the treatment of IDH1 mutated tumors, including brain tumors.

To date, a number of mutant IDH1 inhibitors have been reported and only **IDH-305** (structure not disclosed), **FT-2102** (structure not disclosed), **BAY-1436032** and **AG-120** have entered into clinical trials, of which **AG-120** in Phase III clinical trial.[11,12] According to the binding modes, the active sites of mIDH1 inhibitors could be classified as four types: substrate-based pocket, Seg-2 allosteric pocket, Mg^{2+} pocket and other type. **SYC-435** (**1**), a representative agent based on substrate-based pocket (*Ki* = 120 nM), reveals a significant selectivity for mIDH1 over WT-IDH1.[13] **GSK321** (**2**), binding to the Seg-2 allosteric pocket and locking the mutant proteins into a catalytically inactive "open" conformation to prevent mIDH1 turnover, inhibits the D-2-HG production and the growth and differentiation of mIDH1 cells.[14,15] **VVS** (**3**), with IC_{50} values of 11 nM against IDH1-R132H and 259 nM against IDH1-R132C, is noncompetitive with NADPH and can selectively inhibit mIDH1 via direct interaction with Asp279 in the metal-binding pocket.[16] **AGI-5198** (**4**), developed by Agios Pharmaceuticals, is a potent and selective mIDH1 inhibitor that reduces levels of D-2-HG in tumor cells with the IC_{50} values of 70 nM and 160 nM against IDH1-R132H and IDH1-R132C, respectively.[17,18] **AG-120** (**5**, IC_{50} = 8 nM) is under a Phase III clinical trial and early evidence suggested it is well tolerated with majority of mild to moderate adverse events.[11,12] **BAY-1436032** (**6**) is a novel pan-inhibitor of IDH1 with different codon 132 mutations.[19] Recently, it has been entered into Phase I to evaluate its safety, tolerability, pharmacokinetics, and pharmacodynamics in patients with IDH1-R132 mutated tumors.[20,21] **BRD-2879** (**7**) and **8**, discovered by high-throughput screen (HTS) and further optimization, are potent and selective mIDH1 inhibitors.[22,23] Notably, Novartis reported a novel class of mIDH1 inhibitors with 3-pyrimidin-4-yl-oxazolidin-2-ones motif, such as **NI-1** (**9**, IC_{50} = 0.094 μM, IDH1-R132H).[24] In this article, to obtain more active and drug-like compounds with new scaffolds, we used **NI-1** as a template compound and designed a series of 3-pyrazine-2-yl-oxazolidin-2-ones. Interestingly, during the period of biological evaluation of our target compounds, **IDH889** (**10**, IC_{50} = 20 nM), an analog of **NI-1**, was reported its co-crystal complex with IDH1-132H.[25] The disclosed structures of **1**–**10** are presented (Fig. 1).

Based on the analysis of these findings, we successfully designed and synthesized a series of 3-pyrazine-2-yl-oxazolidin-2-ones allosteric mIDH1 inhibitors. All compounds display inhibitory activity against D-2-HG production in tumor cells harboring IDH1-R132H and IDH1-R132C at 10 μM and 50 μM. The most active one **3g** displays weak inhibition against WT-IDH1, indicating its good selectivity for mIDH1 inhibition. Molecular docking was also studied to predict the binding mode of **3g** at the allosteric site of mIDH1. Importantly, **3g** can effectively cross BBB in PAMPA model. Taken together, our work identified 3-pyrazine-2-yl-oxazolidin-2-ones as a novel class of selective and potent allosteric mIDH1 inhibitors, and **3g** deserves further optimization as an anti-tumor agent to treat patients with IDH1 mutated brain cancers.

2. Results and discussion

2.1. Chemistry

The general route for the synthesis of the target compounds **3a–3m** is depicted in Schemes 1. It started from 2,6-dichloropyrazine, selectively substituted by (*S*)-4-isopropyloxazolidin-2-one at an ice bath to afford the intermediate **I**. (*S*)-1-(4-bromophenyl) ethan-1-amine was reacted with **1a–1m** under $Pd[P(Ph)_3]_4/K_2CO_3$ at 80 °C to afford the intermediates **2a–2m**. Then **I** was reacted with **2a–2m** using tris(dibenzylideneacetone)dipalladium and BINAP as the catalysts in anhydrous toluene at 90 °C under nitrogen to give the target compounds **3a–3m**.

2.2. Biological evaluation

*Effects of **3a–3m** on D-2-HG production in HEK-293T cells expressing IDH1-R132H and IDH1-R132C.* First, we employed HEK-293T cell lines expressing IDH1-R132H and IDH1-R132C to test the inhibitory effects of **3a–3m** at two concentrations (10 μM and 50 μM). **NI-1**, a known mIDH1 inhibitor, was chosen as a positive control. Then the D-2-HG concentration in the cells was measured after 3 days of incubation (Table 1). To our surprise, all the tested compounds could inhibit the D-2-HG production in cells carrying IDH1-R132H or IDH1-R132C at 50 μM (Fig. 2). **3b**, **3g**, **3j** and **3k** showed relatively strong inhibitory effects at both 10 μM and 50 μM. However, **3c**, **3d**, **3f**, **3h** and **3l** exhibited relatively weak inhibitory effects on the production of D-2-HG at 10 μM. As with **NI-1**, **3g** and **3k** strongly reduced the D-2-HG levels in the cells than other tested compounds at the two concentrations, demonstrating that the compounds with 3-pyrazine-2-yl-oxazolidin-2-ones have a bit better inhibitory effects on mutant IDH1 and deserve further optimization.

Cytotoxicity towards HEK-293T WT-IDH1. Owning weak or no effects on WT-IDH1 is essential for an ideal mIDH1 inhibitor. Thus, all the compounds were tested to verify the selectivity of mIDH1 over WT-IDH1. Unexpectedly, **3b**, **3k** and **3m** exhibited relatively strong toxicity compared to other tested compounds at 50 μM. However, **3a** and **3h** were found to retain a better selectivity between mIDH1 and wt-IDH1 at 10 μM and 50 μM. Importantly, **3g** had a controlled toxicity with the values of 89.8% at 10 μM and 74.5% at 50 μM. All the compounds showed no significant toxicity at 10 μM (Fig. 3).

PAMPA-BBB assays. Blood-brain barrier (BBB) permeability is essential for successful central nervous system (CNS) drugs. To measure the possible BBB permeability of **3g** in *vivo*, the parallel artificial membrane permeation assay of the blood-brain barrier (PAMPA-BBB) was performed. On the basis of the measured permeability, **3g** could cross the BBB (Table 2).

2.3. The preliminary structure-activity relationship (SAR) analysis

As shown in Table 1, when R group is the phenyl ring, different substituents are introduced in all positions of R, which has a significant effect on the inhibitory activities of the compounds. For the *para*-position, **3b** (with -F) and **3k** (with $-CF_3$) perform much better, while for meta- and *ortho*-position, **3g** (with $-OCH_3$) and **3i–3j** (with $-CH_3$) have better inhibitory activities, suggesting that electron-withdrawing groups in the *para*-position or electron-donating substituents in the meta- and *ortho*-position are more favorable for R. For the effects of R group's size on the activity, **3m** and **3a** exhibits relatively stronger reduction of D-2-HG levels compared to **3l**, indicating that smaller groups are more suitable for R, but larger groups are limited in this cavity.

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