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Identification of novel allosteric inhibitors of mutant isocitrate dehydrogenase 1 by cross docking-based virtual screening

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ABSTRACT

IDH1 mutation (mIDH1) occurs in 20–30% of gliomas and is a promising target for the cancer therapy. In this article, a cross docking-based virtual screening was employed to identify seven small molecules for the allosteric site of mIDH1. Compounds **ZX01**, **ZX05** and **ZX06** exhibited the potent inhibitory activity and the high selectivity against WT-IDH1, providing a good starting point for the further development of highly selective mIDH1 inhibitors. Importantly, the parallel artificial membrane permeation assay of the blood-brain barrier (PAMPA-BBB) identified **ZX06** with a good ability to penetrate BBB. These findings indicate that **ZX06** deserves further optimization as a lead compound for the treatment of patients with IDH1 mutated brain cancers.

Isocitrate dehydrogenase 1 (IDH1), a key enzyme in the biosynthesis, catalyzes oxidative decarboxylation of isocitrate acid to α-ketoglutaric (α-KG) using divalent magnesium ion and $NADP^+$ (or NAD^+) as cofactors.[1] However, recent studies reveal that IDH1 is a metabolic enzyme associated with the progression of several tumors, including gliomas, acute myeloid leukemia and other solid tumors.[2,3]

Mutations have been frequently found in IDH1 R132H, IDH2 R140Q and IDH2 R172K in several cancer types, including up to 70% of low grade and secondary gliomas and up to 10% of acute myeloid leukemia.[4] The most common mutation in IDH1 is a key amino acid residue Arg132 located in the active site, in which R132H is the predominant.[5] Specific mutations are heterozygous missense mutations and result in a neomorphic catalytic activity of IDH1, which is the conversion of α-KG to D2-hydroxyglutarate (D2-HG).[6,7] D2-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 D2-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 noted that mutant IDH1 is a promising drug target for the treatment of IDH1 mutated tumors, especially brain tumors.

However, developing allosteric inhibitors of mIDH1 remains challengs because few known allosteric sites of mutant IDH1 and ligands have been reported yet.[11] Herein, we explored the allosteric sites including previously reported Mg^{2+} pocket and Seg-2 pocket of mIDH1.[12,13] The two sites are functionally conserved

Abbreviations: IDH, isocitrate dehydrogenase; ICT, isocitrate acid; α-KG, α-ketoglutaric acid; D2HG, D-2-hydroxyglutaric acid; mIDH1, mutant IDH1; mIDH2, mutant IDH2; R132H, Arg132 mutation to His; R132C, Arg132 mutation to Cys; WT, wild type; R140Q, Arg140 mutation to Gln.

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across such evolutionarily IDH1 as IDH1 R132H and IDH1 R132C.[12,13] Of the reported IDH1 R132H inhibitors, some have related crystallographic data and may be separated into two distinct classes: 1) that occupy the active sites such as **1** and **2** (Fig. 1), and 2) that occupy the remote allosteric sites and then render the mutant protein inactive through conformational changes, such as **3** (**VVS**) and **4** (**GSK 321**).[12–14]

In 2016, our joint group conducted an optimal docking-based screening of approximately 200 000 compounds and evaluate coulpes against the HEK-293T cells transfected with IDH1 R132H and IDH1 R132C.[15] Interestingly, compound **FX-03** possesses a high selectivity against WT-IDH1, but it was discontinued due to its poor potency (IC_{50} = 50 μM).[15]

In order to discovery more potent mIDH1 inhibitors, we employed cross docking at mIDH1's two allosteric sites (Mg^{2+} and Seg-2 pockets), combined with subsequent molecular docking for selectivity over mIDH2, and PAINS filtering to identify active hits with novel skeletons (Fig. 2). To the best of our knowledge, the cross docking at two allosteric sites in this article was firstly reported. The most active one **ZX06** was a novel and potent compound with weak inhibition against WT-IDH1 and modest micromolar inhibitory activity against HEK-293T cells transfected with IDH1 R132H or IDH1 R132C. Molecular docking was also studied to predict the binding mode of **ZX06** at the two allosteric sites of mIDH1. Importantly, **ZX06** can effectively penetrate BBB in PAMPA-BBB model. Taken together, our work identified cross docking-based virtual screening as a practice tool for further discovery of more potent and selective mutant IDH1 inhibitors, and **ZX06** deserves further optimization as an antitumor agent to treat patients with IDH1 mutated brain cancers.

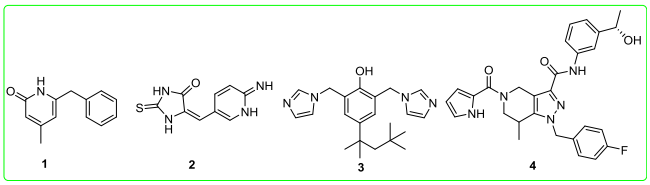

Fig. 1. Representative allosteric mIDH1 inhibitors.

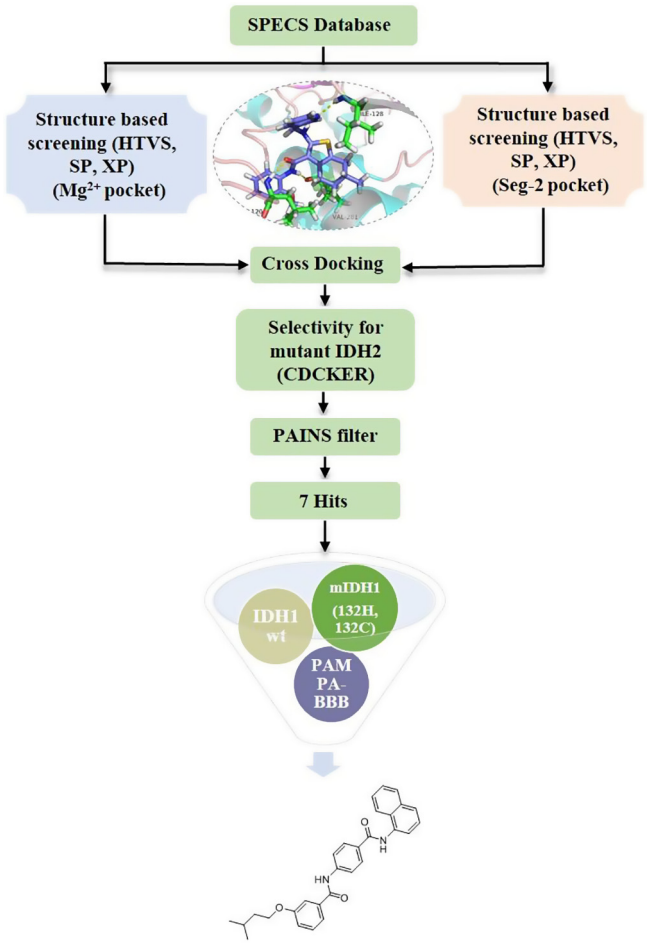

Fig. 2. The flow diagram of the molecular modeling protocol.

The conformation of the Seg-2 pocket in IDH1 R132H homodimer depends on the bound ligand. Specially, the key residues (such as Val281, Gly284 and Tyr285) in the Seg-2 pocket are disordered in the open IDH1-$NADP^+$ binary complex, but it acquires a partial helical conformation in the closed IDH1-$NADP^+$-α-KG ternary complex. It suggests that Seg-2 pocket undergoes a loop-to-helix transition during the mIDH1 catalytic cycle. Thus, we used a structural model of the Seg-2 pocket to recapitulate this repertoire of ligand-induced conformation. The crystal structure of IDH1 R132H bound to **GSK 321** (Seg-2 pocket, PDB code: 5DE1) and **VVS** (divalent magnesium pocket, PDB code: 4UMX) were employed for molecular docking. The difference of two allosteric sites were shown in Fig. 3. The SPECS database (http://www.specs.net) containing 198 745 compounds was used as the chemical library. First, the compounds with unfavourable physicochemical properties were filtered out using Pipeline Pilot 7.5. The remaining compounds were prepared with Ligprep to generate all stereoisomers and different protonation states by Epik. The prepared ligands were docked into the two allosteric sites with the Glide module in Schrodinger by Cross Docking technique. The docking procedure was validated by reproducing the **GSK 321** binding mode with a root-mean-square deviation (RMSD) of 0.32 Å.

The candidate molecules were picked out on the basis of the following considerations. 1) The binding poses should be reasonable. Those molecules with high Glide G_Score strain energy or not occupying the two allosteric sites were not picked out for further validation. 2) Those molecules that can form three distinguishable hydrogen bonds with Leu120, Ile128, Val281 or Gly284, and produce π-π stacking interactions with Arg119, were considered as the candidates for the next step. The reason is that these residues can produce key interactions with **GSK 321** in the Seg-2 pocket (Fig. 4).[14] 3) Those molecules should make direct interaction with the metal-binding Asp279 in divalent magnesium pocket. Consequently, nine molecules were chosen for subsequent selectivity docking (Table 1 in the Supplementary Data).

Given Q316 in two subunits may be involved in the specific recognition of **AGI-6780** by IDH2 R140Q (PDB code: 4JA8, Fig. 5),[16] we chose this residue as a hydrogen bond constraint during the CDOCKER procedure. Seven compounds performed poorly, with the molecular orientations and extensive hydrogen bond interactions in mIDH2, which were much worse than the initial ligand **AGI-6780**. After removal of the PAINS compounds online, we purchased seven compounds (**ZX01**–**ZX07**) for cellular assays (Fig. 6).

To gain a better understanding of the molecular basis of the IDH1 R132H or IDH1 R132C cellular inhibitory activity, we analyzed the putative binding modes of **ZX01**, **ZX05** and **ZX06** in two allosteric sites. As depicted in Fig. 7 (Seg-2 pocket), the 3-nitrogen of pyridine ring of **ZX01** is capable of forming hydrogen bond with Ile128. The amide linker form two strong hydrogen bonds with Leu120 and Val281, with bond lengths of 2.1 Å and 1.8 Å, respectively. If **ZX01** could form polar interactions with Gly284, its binding affinity with IDH1 R132H will be enhanced.