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Design and synthesis of novel 2-arylbenzimidazoles as selective mutant isocitrate dehydrogenase 2 R140Q inhibitors

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Abstract

A series of novel 2-arylbenzimidazoles have been designed, synthesized and evaluated for their inhibitory activity against IDH2 R140Q mutant. The preliminary results indicated that four compounds **7b**, **7c**, **7m** and **7r** displayed the potent inhibitory activity against IDH2 R140Q mutant. Among them, compound **7c** showed the highest inhibitory activity, with the IC_{50} value of 0.26 μM, which was more active than positive control enasidenib. The exquisite selectivity of **7c** for IDH2 R140Q mutant isoform was demonstrated by the poor activity against the IDH1 R132C mutant, IDH1 R132H mutant, wild-type IDH1, IDH2 R172K mutant and the wild-type IDH2.

Keywords: 2-Arylbenzimidazoles; Synthesis; IDH2 R140Q; Inhibitors

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Isocitrate dehydrogenase (IDH) is one of the metabolic enzymes most closely associated with cancer risk and clinical outcome.[1] In the tricarboxylic acid cycle, IDH catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) using Mg^{2+} and $NADP^+$ (or NAD^+) as cofactors, participating in various molecular processes including histone and DNA modifcations.[2-6] Heterozygous point mutations in the active site arginine residues of IDH1 (R132) and IDH2 (R140 and R172) are observed in cancers including low-grade gliomas, secondary glioblastomas, angioimmunoblastic T-cell lymphomas, acute myeloid leukemia (AML) and so on.[7-11] Approximately 10-40% of patients with AML carry mutations in the IDH1/2 gene, making the two proteins promising therapeutic targets in AML.[4, 12] In AML patients with IDH2 mutations, R140 mutations occur in approximately 80% of patients as compared to R172 mutations that occur in only 20%.[13]

IDH2 mutations confer a gain-of-function and lead to neomorphic enzymatic activity of the mutated IDH enzyme.[14] Instead of catalyzing the conversion of isocitrate to α-KG, R172 and R140 mutant IDH2 both catalyze the conversion of α-KG to 2-hydroxyglutarate (2-HG) (**Fig. 1**).[15] Supra-normal levels of intracellular 2-HG lead to hypermethylation of target genes and a block in cellular differentiation.[16] Currently, the IDH2 R140Q inhibitor enasidenib has been approved by FDA for the clinical treatment of IDH2 mutation-positive AML (**Fig. 2**), which opens up a new avenue for the therapy of AML patients. Therefore, it is essential to develop novel IDH2 R140Q inhibitors with improved potency and exquisite selectivity.

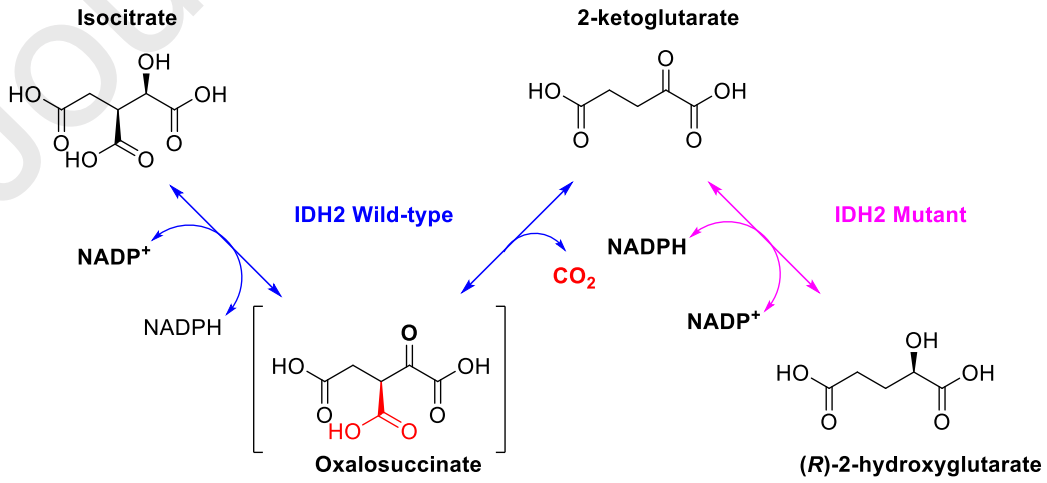


Fig. 1 Reactions catalyzed by wild-type and mutant IDH2.

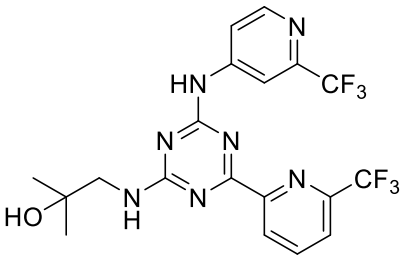


Fig. 2 Chemical structure of Enasidenib approved by the FDA for AML treatment.

Treatment with AGI-6780 induced differentiation of TF-1 erythroleukemia and primary human AML cells in vitro. These data provide proof-of-concept that inhibitors targeting mutant IDH2 R140Q could have potential applications as a differentiation therapy for cancer.[16] However, the selectivity of AGI-6780 for inhibiting IDH2 R140Q is not ideal. Due to the cocrystal structure of IDH2 R140Q/AGI-6780 reported,[16] it is possible to develop novel IDH2 R140Q inhibitors through structure-based drug design. In this study, we take AGI-6780 as a lead compound, and scaffold hopping strategy was used to design a novel series of 2-arylbenzimidazoles as IDH2 R140Q mutant inhibitors (**Fig. 3**). In the designed compounds, various groups were introduced to investigate the structure-activity relationship (SAR).

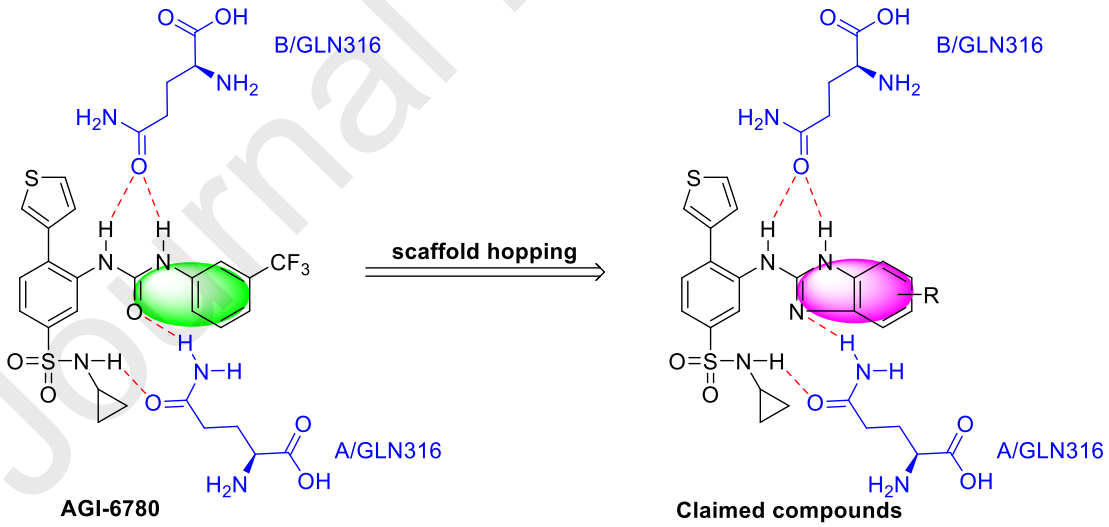
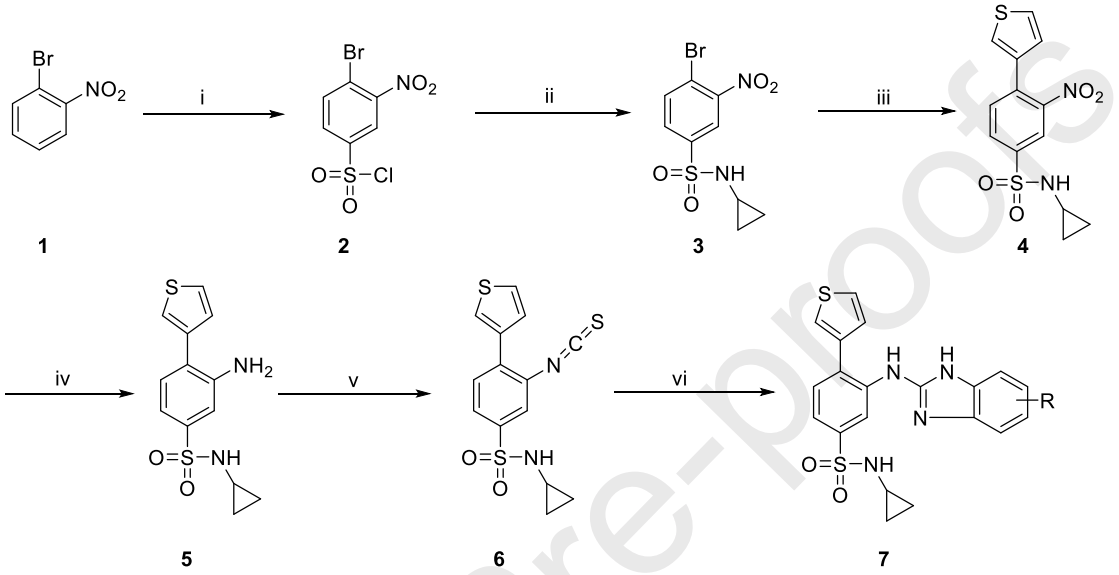


Fig. 3 Design strategy of novel 2-arylbenzimidazoles IDH2 R140Q inhibitors.

Chlorosulfonylation of commercially available 2-nitrobromobenzene (**1**) to obtain intermediate (**2**), followed by reaction with cyclopropylamine afforded the cyclopropylsulfonamide (**3**). Palladium catalyzed cross-coupling of the aryl bromide with 3-thiopheneboronic acid yielded the biaryl sulfonamide (**4**). Reduction of the aryl-

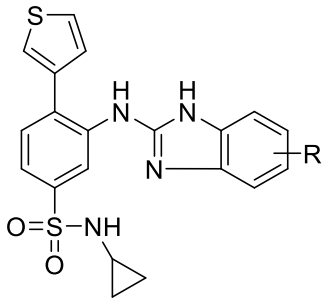
nitro group with iron yielded the aniline (**5**), which was reacted with di-2-pyridyl thionocarbonate to afford the isothiocyanate (**6**). The title compounds **7a-t** were prepared by the condensation of the isothiocyanate **6** with various substituted *o*-phenylenediamines, respectively (**Scheme 1**). The spectral data for all the synthesized compounds are provided in the supporting information.



Scheme 1 Reagents: (i) chlorosulfonic acid, 120°C ; (ii) cyclopropylamine, DIEPA, dichloromethane, room temperature; (iii) 3-thiopheneboronic acid, Pd_2Cl_2(dppf), cesium carbonate, dioxane, 100°C ; (iv) ammonium chloride, iron powder, methanol, H_2O, 80°C, 5 h; (v) di-2-pyridyl thionocarbonate, acetonitrile, overnight, 40 ºC; (vi) substituted *o*-phenylenediamine, room temperature.

Setting AGI-5198, AGI-6780 and enasidenib as the positive controls, all the synthesized compounds were evaluated for their abilities to inhibit the IDH2 R140Q mutant in an enzyme-based assay. The results were expressed in IC_{50}, which represented the concentration of the tested compounds for 50% IDH2 R140Q mutant inhibition. The selectivity of the derivatives with IC_{50} values less than 1 μM were then tested for their inhibitory activity against IDH1 R132C mutant, IDH1 R132H mutant, wild-type IDH1, IDH2 R172K mutant and the wild-type IDH2.

Table 1 In vitro IDH2 R140Q mutant inhibitory activities



Compd	R	IC_{50} (μM)
7a	H	5.41±0.31
7b	2-Cl	0.73±0.09
7c	2-Br	0.26±0.04
7d	2-NO_2	3.63±0.51
7e	3-F	11.89±4.57
7f	3-Br	2.02±0.13
7g	3-OMe	46.94±5.09
7h	3-NO_2	81.00±15.14
7i	3-CN	97.87±18.02
7j	3-CF_3	14.09±4.68
7k	3-COOMe	>100
7l	3-COOEt	>100
7m	2,4-2Me	0.96±0.07
7n	2,4-2F	4.94±0.30
7o	3,4-2Me	1.61±0.09
7p	3,4-2F	13.80±2.14
7q	3,4-2Cl	2.72±0.16
7r	3,4-2Br	0.65±0.09
7s	3-F,4-Cl	10.35±2.03
7t	3-CF_3,4-Cl	4.34±0.25
AGI-6780		0.06±0.02
Enasidenib		0.31±0.09

In general, from the obtained data, it was observed that some of the 2-arylbenzimidazoles, **7b**, **7c**, **7m** and **7r** displayed remarkable IDH2 R140Q mutant inhibitory activity. Particularly, the compound **7c** showed the highest inhibitory activity against IDH2 R140Q mutant, with the IC_{50} value of 0.26 μM, which was more active than positive control enasidenib.

The SARs analysis showed that the nature of the substituents greatly influenced the IDH2 R140Q mutant inhibitory activity of these compounds. In the monosubstituted series, the inductive effects of substituents can significantly reflect active sequence of the derivatives. It followed the rules: the weaker the negative inductive effect is, the

stronger the activity is, e.g. **7c** (2-Br) > **7b** (2-F) and **7f** (3-Br) > **7e** (3-Cl) > **7j** (3-CF_3) > **7h** (3-NO_2). Moreover, the halogen disubstituted series also followed the rules, e.g. **7r** (3,4-2Br) > **7q** (3,4-2Cl) > **7p** (3,4-2F).

In the disubstituted series, when substituents were electron-donating methyl groups, they were more active than electron-withdrawing fluoro-substituted derivatives, e.g. **7m** (2,4-2Me) > **7n** (2,4-2F) and **7o** (3,4-2Me) > **7p** (3,4-2F). However, electron-donating methyl-substituted derivatives were less active than weak electron-withdrawing bromo-substituted derivatives, e.g. **7r** (3,4-2Br) > **7o** (3,4-2Me), illustrating that the induction effect of substituents greatly affects the activity of compounds.

Table 2 Selectivity profiling of potent 2-arylbenzimidazoles

Compd	IDH1 WT IC_{50} (μM)	IDH1 R132C IC_{50} (μM)	IDH1 R132H IC_{50} (μM)	IDH2 WT IC_{50} (μM)	IDH2 R140Q IC_{50} (μM)	IDH2 R172K IC_{50} (μM)
7b	>100	>100	>100	>100	0.73±0.09	78.8
7c	>100	>100	>100	>100	0.26±0.04	>100
7m	>100	>100	>100	>100	0.96±0.07	51.8
7r	>100	>100	>100	>100	0.65±0.09	>100
AGI-5198	>100	0.39±0.07	0.17±0.01	>100	>100	>100
AGI-6780	>100	>100	>100	>100	0.06±0.02	10.39±1.61
Enasidenib	>100	>100	>100	>100	0.31±0.09	1.08±0.23

As the SAR investigation revealed functional group modifications that provided potent inhibitors in the IDH2 R140Q mutant enzymatic assay, we selected a focused set of analogs for evaluation against IDH1 R132C mutant, IDH1 R132H mutant, wild-type IDH1, IDH2 R172K mutant and wild-type IDH2. As shown in Table 2, **7b**, **7c**, **7m** and **7r** showed exquisite selectivity for IDH2 R140Q mutant subtype and was demonstrated by the poor activity against the IDH1 R132C mutant, IDH1 R132H mutant, wild-type IDH1, IDH2 R172K mutant and the wild-type IDH2.

To examine the possible binding mode between **7c** and IDH2 R140Q, docking analysis was performed using Autodock vina 1.1.2[17] and the results were shown in **Fig. 4**. The maximum binding affinity between **7c** and the IDH2 R140Q was predicted to be -6.8 kcal/mol. In comparison with AG-6780, the binding mode of **7c** was similarly based on the reported crystal structure of AG-6780 (**Fig. 4A**). The 3-thienyl group of

the compound **7c** located at the hydrophobic pocket, surrounded by the residues B/Val-297, B/Trp-306 and B/Ile-319, while cyclopropane ring of the compound **7c** stretched into another hydrophobic pocket that consisted of A/Leu-160, A/Trp-164, A/Val-297, A/Leu-298 and A/Leu-320, forming a strong hydrophobic binding (**Fig. 4B**). Detailed analysis showed that the benzimidazole scaffold of the **7c** formed anion-π interactions with the residues A/Asp-312 and B/Asp-312, respectively. Importantly, the four key hydrogen bond interactions was observed between the **7c** and the residues A/Gln-316 (bond lengths: 2.0 and 3.5 Å) and B/Gln-316 (bond lengths: 2.8 and 3.2 Å), which was the main interaction between the **7c** and the IDH2 R140Q (**Fig. 4B**). All these interactions helped **7c** to anchor in the binding site of IDH2 R140Q. In summary, the above molecular simulations give us rational explanation of the interactions between the **7c** and the IDH2 R140Q, which provided valuable information for the development of the IDH2 R140Q inhibitors.

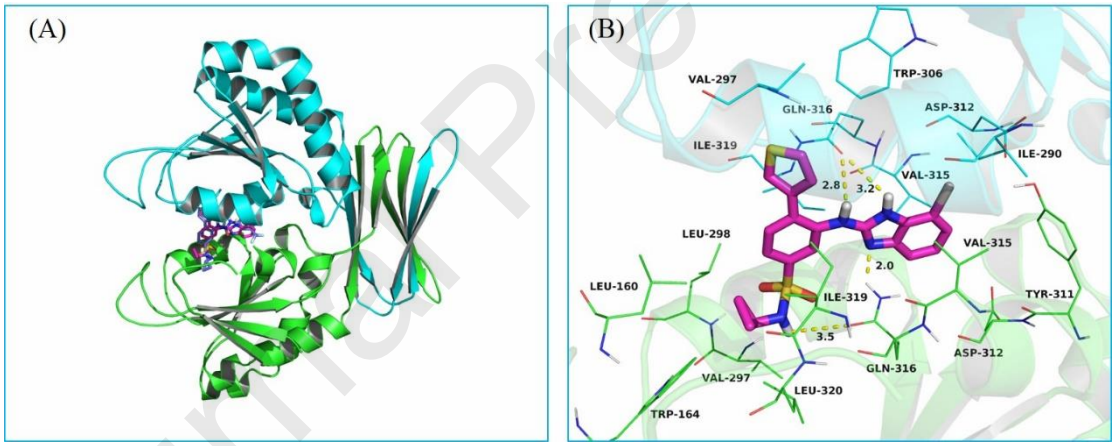


Fig. 4 Molecular docking simulation of the interactions between **7c** and IDH2 R140Q. (A) Compound **7c** was docked into the binding site of IDH2 R140Q with AGI-6780 overlapped (total view). (B) Detailed view of the binding mode between **7c** and IDH2 R140Q. IDH2 R140Q was represented with cartoon (chain A was colored with green, and chain B was colored with cyan), and the representative binding residues were shown in lines; **7c** and AGI-6780 were represented with rose red and violet sticks, respectively. The hydrogen bonds were shown as yellow dotted lines.

In conclusion, a series of novel 2-arylbenzimidazoles were designed, synthesized and biologically evaluated for their inhibitory activity against IDH2 R140Q mutant. Compounds **7b**, **7c**, **7m** and **7r** showed exquisite selectivity for IDH2 R140Q mutant

subtype and demonstrated poor activity against the IDH1 R132C mutant, IDH1 R132H mutant, wild-type IDH1, IDH2 R172K mutant and the wild-type IDH2. Particularly, the compound **7c** showed the highest inhibitory activity against IDH2 R140Q mutant, with the IC_{50} value of 0.26 μM. Taken together, our findings in the present study provide valuable information on novel structures, which presents a most promising lead for further investigation.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

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Highlights

- Synthesis of novel IDH2 R140Q inhibitors with benzimidazole scaffold *via* six steps reaction.
- Selectivity of **7c** for IDH2 R140Q mutant was the best among the twenty compounds.
- **7c** might be a lead compound deserved further structural optimization.

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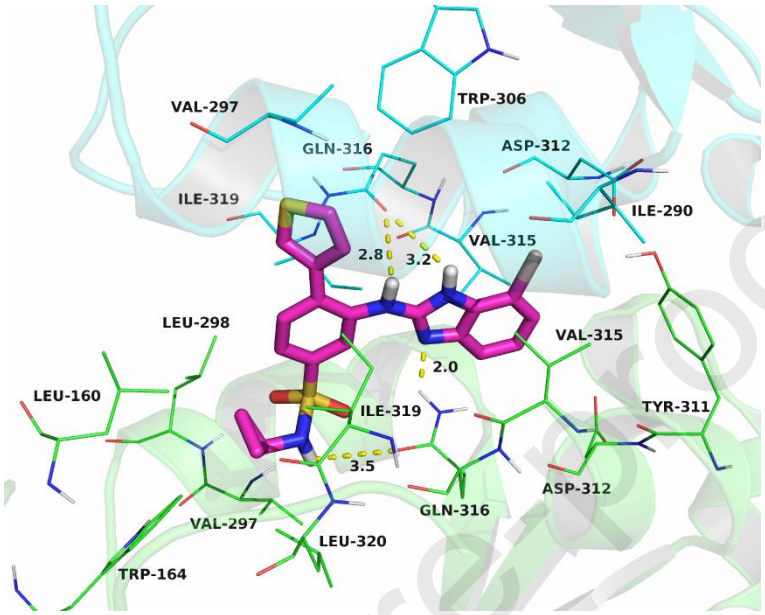
Graphical abstract

The exquisite selectivity of **7c** for IDH2 R140Q mutant isoform was demonstrated by the poor activity against the IDH1 R132C mutant, IDH1 R132H mutant, wild-type IDH1, IDH2 R172K mutant and the wild-type IDH2.



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