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Synthesis and evaluation of radiolabeled AGI-5198 analogues as candidate radiotracers for imaging mutant IDH1 expression in tumors

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ABSTRACT

Mutations in the metabolic enzyme isocitrate dehydrogenase 1 (IDH1) are commonly found in gliomas. AGI-5198, a potent and selective inhibitor of the mutant IDH1 enzyme, was radiolabeled with radioiodine and fluorine-18. These radiotracers were evaluated as potential probes for imaging mutant IDH1 expression in tumors with positron emission tomography (PET). Radioiodination of AGI-5198 was achieved using a tin precursor in 79 ± 6% yield (n = 9), and ^{18}F-labeling was accomplished by the Ugi reaction in a decay-corrected radiochemical yield of 2.6 ± 1.6% (n = 5). The inhibitory potency of the analogous non-radioactive compounds against mutant IDH1 (IDH1-R132H) was determined in enzymatic assays. Cell uptake studies using radiolabeled AGI-5198 analogues revealed somewhat higher uptake in IDH1-mutated cells than that in wild-type IDH1 cells. The radiolabeled compounds displayed favorable tissue distribution characteristics in vivo, and good initial uptake in IDH1-mutated tumor xenografts; however, tumor uptake decreased with time. Radioiodinated AGI-5198 exhibited higher tumor-to-background ratios compared with ^{18}F-labeled AGI-5198; unfortunately, similar results were observed in wild-type IDH1 tumor xenografts as well, indicating lack of selectivity for mutant IDH1 for this tracer. These results suggest that AGI-5198 analogues are not a promising platform for radiotracer development. Nonetheless, insights gained from this study may help in design and optimization of novel chemical scaffolds for developing radiotracers for imaging the mutant IDH1 enzyme.

Malignant gliomas frequently harbor mutations in the gene encoding for the metabolic enzyme isocitrate dehydrogenase 1 (IDH1).[1] Studies suggest that IDH1 mutations contribute to epigenetic dysregulation and widespread metabolic changes in tumor cells, including depletion of key cellular biochemicals such as glutamine and glutamine-derived metabolites.[2,3] Most of these effects are mediated by the metabolite D-2-hydroxyglutarate (D-2-HG), which is generated by mutated IDH1 and accumulates in tumor cells at levels ~100-fold higher than those found in wild-type cells.[4,5] IDH1 mutations impair the normal catalytic function of the enzyme – its ability to convert isocitrate to α-ketoglutarate (α-KG) – and confer a new function that enables the mutant IDH1 to recognize α-KG as a substrate and reduce it to D-2-HG.[1,4] IDH1 mutations are present in up to 80% of World Health Organization (WHO) grades II and III gliomas and in secondary glioblastoma, but are seldom found in primary (*de novo*) glioblastoma.[1,6] IDH mutations are also present in several other cancers including acute myeloid leukemia (AML),[7] intrahepatic cholangiocarcinoma,[8] Ollier disease and Maffucci syndrome,[9] suggesting a causative role played by IDH mutations in cancers.

Over the past few years, IDH1 mutations (IDH1-R132H, IDH1-R132C) have emerged as novel and highly promising drug targets in gliomas. Several pharmaceutical companies have active drug development programs targeting IDH1 mutations, and most of these programs are based on small-molecule IDH1 inhibitors representing a variety of chemical scaffolds.[10] Preclinical studies using prototypical compounds from some of these chemical classes have established that small-molecule inhibitors can bind to the mutant IDH1 enzyme and inhibit its ability to produce D-2-HG, thereby reversing the effects of IDH mutations on cancer cells that harbor these mutations.[11] Compounds based on a phenyl-glycine chemical scaffold were among the first chemical analogues that were

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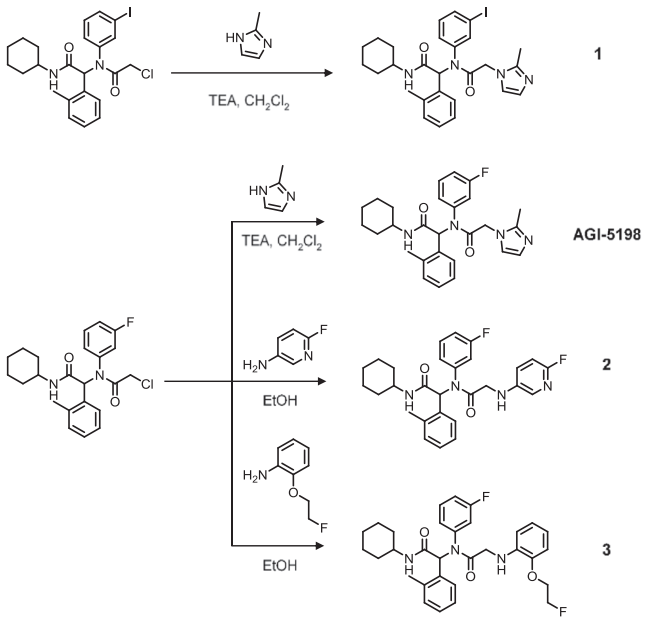
identified as potent inhibitors of mutant IDH1 enzymes. High-throughput screening identified compounds with half-maximal inhibitory concentration (IC_{50}) values of ≤0.1 μM for the most potent analogues against IDH1-R132H, the most commonly occurring IDH-mutation in glioma.[12] The most extensively studied compound from the phenyl-glycine series, AGI-5198 (IC_{50}: 0.07 μM), has been shown to inhibit D-2-HG production and have anti-tumor effects in IDH1-mutated glioma xenografts in vivo.[11,12] AGI-5198 also has very high selectivity for mutant IDH1 vs. wild-type IDH1 indicated by its lack of inhibitory potency against wild-type IDH1 (IC_{50}: >100 μM).[12] With the goal of developing radiolabeled compounds for noninvasive imaging of IDH1 mutations in gliomas with positron emission tomography (PET), we have synthesized radiolabeled analogues of AGI-5198 and evaluated their potential as radiotracers for this purpose. AGI-5198 was labeled with radioiodine or fluorine-18 and the labeled compounds were evaluated for their ability to bind to IDH1-mutated tumor cells in vitro and in vivo.

In addition to its high potency and selectivity for mutant IDH1, AGI-5198 offers additional advantage for radiolabeling – it has a fluorine atom which could be replaced with radioactive halogens, fluorine-18 (^{18}F, $t_{1/2}$: 110 min) or iodine-124 (^{124}I, $t_{1/2}$: 4.2 d), for PET imaging purposes. Structure-activity relationship (SAR) studies of phenyl-glycine analogues originally published by Popovici-Muller et al. showed that replacement of the 2-methylimidazole moiety in AGI-5198 with a phenyl or a heterocyclic function was well-tolerated in terms of inhibitory potency against IDH1-R132H.[12] Thus, in addition to AGI-5198 and its iodo-analogue **1**, compounds **2** and **3** were synthesized to explore the possibility of introducing ^{18}F on a different substituent and also to better understand the SAR for radiotracer development (Scheme 1). For radiolabeling, the fluorine atom in compound **2** could be replaced with ^{18}F by an aromatic nucleophilic substitution reaction on precursors containing a suitable leaving group (e.g., nitro, bromo).[13] Similarly, ^{18}F labeling of compound **4** could be achieved by using a sulfonate precursor or by a fluoroalkylation reaction using [^{18}F]fluoroethyl bromide ([^{18}F]FEtBr) on a phenol precursor.[14] AGI-5198 as well as other nonradioactive reference compounds (**1**–**3**) were synthesized and evaluated in racemic form similar to previously published reports, and as shown in Scheme 1.[12,15] First, the chloroacetamide intermediate bearing a fluoro or iodo function on the meta-position of the phenyl ring was constructed by four-component Ugi reaction starting from 3-fluoroaniline or 3-iodoaniline, respectively. Next, the chloroacetamide intermediates were reacted with either 2-methylimidazole for AGI-5198 and **1**, or the corresponding aromatic amine derivative for **2** and **3** to obtain the final compounds. The synthesis of the chloroacetamide derivatives in the Ugi-reaction was achieved in about 65% yield, and substitution of the chloro function with the corresponding amine analogue in compounds **1**–**3** was accomplished in 59–81% yield.



Scheme 1. Synthesis scheme for the nonradioactive phenyl-glycine analogues.

The inhibitory activity of the unlabeled analogues against IDH1-R132H was evaluated in enzymatic assays as previously described using serial twofold dilutions (n = 12) of the test compounds in the presence of the mutant IDH1 substrate α-KG, and by the diaphorase/resazurin detection system.[14] The inhibitory potency (IC_{50}) and maximal inhibitory capacity of the compounds were derived from the inhibitory response curves for each compound (Fig. 1, Table 1).

Table 1
Structural characteristics and inhibitory potency against R132H mutant IDH1 for the synthesized compounds.

Compound	M. Wt.	clogP	Polar surface area (PSA)	IC_{50} vs. IDH1-R132H (μM)
AGI-5198	462.6	4.7	65.0	0.03
1	570.5	5.7	65.0	<0.01
2	492.6	5.8	73.8	1.39
3	535.6	6.4	70.7	26.31

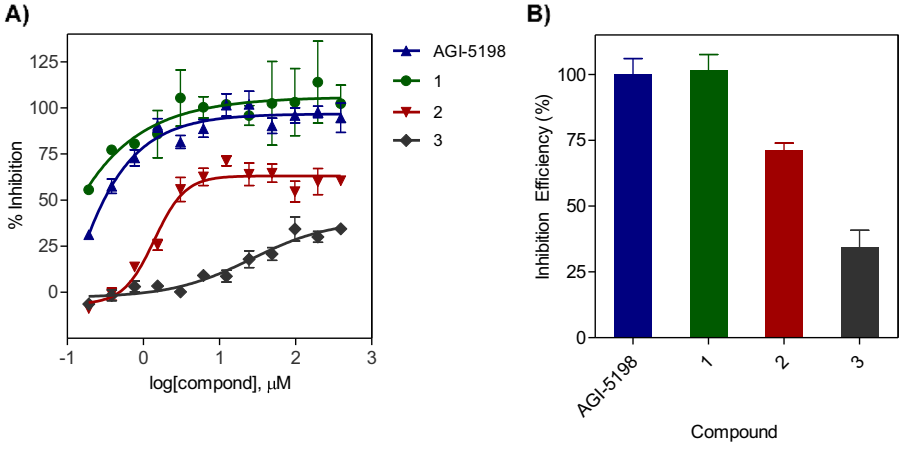


Fig. 1. (A) Inhibitory activity curves for AGI-5198 and the synthesized analogues against IDH1-R132H in enzymatic assays. (B) Inhibitory efficiency shown as the maximal enzyme inhibition observed for the nonradioactive analogues in experiments depicted in (A). Data are presented as mean ± SEM.