European Journal of Medicinal Chemistry 119 (2016) 218–230

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Radiolabeled inhibitors as probes for imaging mutant IDH1 expression in gliomas: Synthesis and preliminary evaluation of labeled butyl-phenyl sulfonamide analogs

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ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form
25 April 2016
Accepted 26 April 2016
Available online 28 April 2016

Keywords:
IDH mutation
Glioma
Mutant IDH1 inhibitor
PET imaging
Fluorine-18
Radioiodine

ABSTRACT

Introduction: Malignant gliomas frequently harbor mutations in the isocitrate dehydrogenase 1 (IDH1) gene. Studies suggest that IDH mutation contributes to tumor pathogenesis through mechanisms that are mediated by the neomorphic metabolite of the mutant IDH1 enzyme, 2-hydroxyglutarate (2-HG). The aim of this work was to synthesize and evaluate radiolabeled compounds that bind to the mutant IDH1 enzyme with the goal of enabling noninvasive imaging of mutant IDH1 expression in gliomas by positron emission tomography (PET).

Methods: A small library of nonradioactive analogs were designed and synthesized based on the chemical structure of reported butyl-phenyl sulfonamide inhibitors of mutant IDH1. Enzyme inhibition assays were conducted using purified mutant IDH1 enzyme, IDH1-R132H, to determine the IC_{50} and the maximal inhibitory efficiency of the synthesized compounds. Selected compounds, **1** and **4**, were labeled with radioiodine (^{125}I) and/or ^{18}F using bromo- and phenol precursors, respectively. In vivo behavior of the labeled inhibitors was studied by conducting tissue distribution studies with [^{125}I]**1** in normal mice. Cell uptake studies were conducted using an isogenic astrocytoma cell line that carried a native IDH1-R132H mutation to evaluate the potential uptake of the labeled inhibitors in IDH1-mutated tumor cells.

Results: Enzyme inhibition assays showed good inhibitory potency for compounds that have iodine or a fluoroethoxy substituent at the *ortho* position of the phenyl ring in compounds **1** and **4** with IC_{50} values of 1.7 μM and 2.3 μM, respectively. Compounds **1** and **4** inhibited mutant IDH1 activity and decreased the production of 2-HG in an IDH1-mutated astrocytoma cell line. Radiolabeling of **1** and **4** was achieved with an average radiochemical yield of 56.6 ± 20.1% for [^{125}I]**1** (n = 4) and 67.5 ± 6.6% for [^{18}F]**4** (n = 3). [^{125}I]**1** exhibited favorable biodistribution characteristics in normal mice, with rapid clearance from the blood and elimination via the hepatobiliary system by 4 h after injection. The uptake of [^{125}I]**1** in tumor cells positive for IDH1-R132H was significantly higher compared to isogenic WT-IDH1 controls, with a maximal uptake ratio of 1.67 at 3 h post injection. Co-incubation of the labeled inhibitors with the corresponding nonradioactive analogs, and decreasing the normal concentrations of FBS (10%) in the incubation media substantially increased the uptake of the labeled inhibitors in both the IDH1-mutant and WT-IDH1 tumor cell lines, suggesting significant non-specific binding of the synthesized labeled butyl-phenyl sulfonamide inhibitors.

Conclusions: These data demonstrate the feasibility of developing radiolabeled probes for the mutant IDH1 enzyme based on enzyme inhibitors. Further optimization of the labeled inhibitors by modifying the chemical structure to decrease the lipophilicity and to increase potency may yield compounds with improved characteristics as probes for imaging mutant IDH1 expression in tumors.

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http://dx.doi.org/10.1016/j.ejmech.2016.04.066
0223-5234/

1. Introduction

Isocitrate dehydrogenase 1 encodes for the IDH1 enzyme, which plays important roles in cellular metabolism, energy production and maintenance of normal redox status in cells. IDH1 is localized in the cell cytoplasm and peroxisomes where it catalyzes the conversion of isocitrate to α-ketoglutarate (α-KG) and simultaneously reduces NADP to NADPH [1]. α-Ketoglutarate serves as a key intermediate in the tricarboxylic acid cycle (TCA) in cells, and as substrate for several important dioxygenase enzymes. NADPH is a key cofactor in the cholesterol and lipid synthesis pathways and also contributes to cell defense against oxidative damage induced by reactive oxygen species (ROS) [2]. Recent genome-wide mutation analysis studies revealed that gliomas frequently harbor mutations in IDH1 and, to a lesser extent, the homologous IDH2 genes [3,4]. IDH1 mutations are present in over 75% of WHO grade 2 and grade 3 gliomas and in secondary gliomas that develop from lower-grade tumors [2,4,5]. IDH mutations are generally associated with better prognosis when compared to glioma patients with wild-type IDH (WT-IDH) tumors of the same histological type [4,6]. The median overall survival of patients with IDH-mutated glioblastoma and anaplastic astrocytoma is approximately 2–3 times longer than for patients whose tumors have WT-IDH1 [4], strongly suggesting the prognostic significance of IDH1 and IDH2 mutations in glioma [7].

Studies suggest that IDH mutations also play significant roles in tumorigenesis. IDH mutation is an early genetic event in the formation of gliomas, and contributes to tumor pathogenesis through multiple mechanisms that are mediated by the neomorphic activity of the mutated IDH1 enzyme [5,8,9]. IDH mutation results in a loss of normal catalytic activity - conversion of isocitrate to α-KG - for the IDH enzyme but also imparts a new gain-of-function that enables the mutant IDH to convert α-KG to 2-hydroxyglutarate (2-HG) in a stereospecific manner (D-2-HG) [10]. Consequently, tumors that are positive for IDH1 mutation have ~100-fold higher levels of D-2-HG compared to tumors with normal IDH1 (WT-IDH1). Excessive D-2-HG in IDH1-mutated cells competitively inhibits several key cellular dioxygenases that are normally dependent on α-KG as a co-factor for their enzyme activity [11]. This competitive inhibition is presumed to be due to the structural similarity of D-2-HG with the α-KG, and results in the impairment of the activity of multiple cellular dioxygenases leading to stabilization and activation of the transcription factor hypoxia-inducible factor-1α (HIF-1α), epigenetic dysregulation leading to histone methylation and DNA hypermethylation, and impairment of normal collagen maturation [12–14]. IDH1 mutations also directly impact the cellular metabolism of small biochemicals such as amino acids, glutathione derivatives and TCA cycle intermediates, suggesting the occurrence of widespread metabolic changes in IDH1-mutated tumor cells [15,16].

In view of these diverse roles and the impact of IDH1 mutations on cellular metabolism and epigenetics, there is a strong rationale for development of noninvasive imaging methods (e.g. PET) to evaluate mutant IDH1 expression in gliomas. Such a method could enable determination of the IDH1 mutation status in glioma patients noninvasively, predict prognosis and assist in the design and implementation of novel therapeutics for patients with malignant brain tumors as well as other cancers that carry these mutations [2,5]. In this work, we have synthesized and evaluated radiolabeled compounds that have inhibitory activity against the most commonly occurring IDH1 mutation subtype, IDH1-R132H [17]. Selected compounds were labeled with radioiodine and/or fluorine-18, and in vitro studies and preliminary in vivo studies were conducted using the labeled inhibitors in order to assess their suitability as probes for mutant IDH1.

2. Results and discussion

2.1. Chemistry

Butyl-phenyl sulfonamide inhibitors of mutant IDH were among the first compounds shown to inhibit mutant IDH1 activity in vitro. From this chemical class, *N*-(4-butylphenyl)-3-(4-(2-methoxyphenyl)piperazine-1-carbonyl)-4-methylbenzenesulfonamide **A** (Fig. 1) was chosen as the reference compound, which has an IC_{50} value of <1 μM against the mutant IDH1-R132H enzyme [17]. A series of nonradioactive iodo- and/or fluorinated analogs of **A** were synthesized for structure–activity relationship studies and to identify potent inhibitors for radiolabeling (Fig. 2). The basic synthesis route involved the reaction of commercially available 5-(*N*-(4-butylphenyl)sulfamoyl)-2-methylbenzoic acid with appropriate phenyl piperazine derivative in the presence of the coupling reagents EDC and HOBt in DMF (Scheme 1) [17]. For the iodophenyl analogs **1** and **2**, the piperazine derivatives 1-(2-iodophenyl)piperazine and 1-(4-iodophenyl) piperazine were synthesized following a literature report [17], and were subsequently coupled to the benzoic acid derivative in 62% yield for **1** and 71% yield for **2**. The fluoroethyl analogs **4** and **5** were synthesized by introduction of a fluoroethoxy function at the *ortho*- or *para*-position of the phenyl ring in the phenyl piperazine moiety. For these reactions, fluoroethoxyphenyl piperazine derivatives were synthesized by using the corresponding phenol piperazine via a 3-step synthesis route [18]. First, the secondary amine function in the piperazine ring was protected with a Boc-group in quantitative yields. Fluoroethylation of the boc-protected 2- or 4-hydroxypheyl piperazine was achieved by using fluoroethyl bromide (FEtBr) in about 75% yield. Removal of the boc protective groups with a mixture of TFA and CH_2Cl_2 (50/50, v/v) yielded the fluoroethoxyphenyl piperazine derivatives in quantitative yields. The synthesized 2- and 4-fluoroethoxyphenyl piperazine analogs were then reacted with the methylbenzoic acid derivative to obtain the fluoroethoxy analogs **4** and **5** in 40–47% yield (Scheme 1). For compound **3**, the iodine atom was introduced in place of the methyl group in the methylbenzoic acid derivative in a two-step synthesis (**9**) and was then coupled to 1-(-2-methoxyphenyl)piperazine in 59% yield (Scheme 2).

Scheme 3 shows the synthesis route for the fluoroalkyl derivatives **6** and **7**. Introduction of the fluorine atom into the side chain in these compounds was achieved by means of a fluoropropyl (**6**) or a fluorobutyl (**7**) function. For these syntheses, the fluoroalkyl aniline derivatives **12a** and **12b** were synthesized first, and were subsequently reacted with 5-(chlorosulfonyl)-2-methylbenzoic acid to obtain **13a** or **13b**. The synthesis of 4-(3-fluoropropyl)aniline **12a** was achieved using commercially available 3-(4-nitrophenyl)propionic acid (structure not shown) where the carboxylic acid function was first reduced to an alcohol using borane in THF to get **10a**. Treatment of the alcohol derivatives **10a** and **10b**

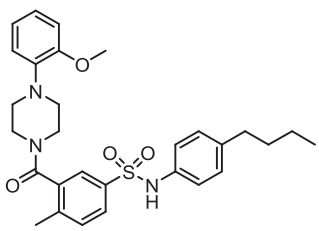

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Fig. 1. Chemical structure of the reference mutant IDH1 inhibitor **A**.