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Inhibition of Mutated Isocitrate Dehydrogenase 1 in Cancer

Fangrui Wu[a], **Gang Cheng**[a], **Yuan Yao**[a], **Mari Kogiso**[b], **Hong Jiang**[a], **Xiao-Nan Li**[b], and **Yongcheng Song**[a]

[a]Department of Pharmacology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, USA.

[b]Department of Pediatrics-oncology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, USA.

Abstract

Background: R132H mutation of isocitrate dehydrogenase 1 (IDH1) are found in ~75% of low-grade gliomas and secondary glioblastomas as well as in several other types of cancer. More chemotypes of inhibitors of IDH1(R132H) are therefore needed.

Objective: To develop a new class of IDH1(R132H) inhibitors as potent antitumor agents.

Method: A biochemical assay was developed to find inhibitors of IDH1(R132H) mutant enzyme. Chemical synthesis and structure activity relationship studies were used to find compounds with improved potency. Antitumor activities of selected compounds were evaluated.

Results: A series of aromatic sulfonamide compounds were found to be novel, potent inhibitors of IDH1(R132H) with K_i values as low as 0.6 μM. Structure activity relationships of these compounds are discussed. Enzyme kinetics studies showed that one compound is a competitive inhibitor against the substrate α-KG and a non-competitive inhibitor against the cofactor NADPH. Several inhibitors were found to have no activity against wild-type IDH1, showing a high selectivity. Two potent inhibitors exhibited strong activity against proliferation of BT142 glioma cells with IDH1 R132H mutation, while these compounds did not significantly affect growth of glioma cells without IDH1 mutation.

Conclusion: This novel series of IDH1(R132H) inhibitors have potential to be further developed for the treatment of glioma with IDH1 mutation.

Keywords

Isocitrate dehydrogenase; Oncogenic mutation; Enzyme inhibitor; Medicinal chemistry

CORRESPONDING AUTHOR: Yongcheng Song, Address: Department of Pharmacology, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030. Tel: 713-798-7415. ysong@bcm.edu.

Conflict of Interest

The author(s) confirm that this article content has no conflicts of interest.

INTRODUCTION

Point mutations of isocitrate dehydrogenase (IDH) 1 and 2, located in cytoplasm and mitochondria, respectively, have been frequently found in cancer including glioma, leukemia and sarcomas. [1–6] In particular, mutations of IDH1 have been identified in ~75% low-grade gliomas (grade II and III), [2,3] which grow slowly but eventually develop to become secondary glioblastoma multiforme (GBM), which is highly invasive grade IV glioma with a very low 5-year survival rate of <10%. R132H (Arg132His) is the predominant (~90%) form of mutation in gliomas. There is therefore a pressing need to find effective treatments for GBM.

IDH is one of the key enzymes in tricarboxylic acid cycle for aerobic metabolism of carbohydrates and fats. Biochemically, IDH catalyzes oxidative decarboxylation of isocitric acid to produce α-ketoglutaric acid (α-KG) (Figure 1A). Mutant IDH enzymes including IDH1(R132H) almost lose the function of the wild-type (WT) enzyme. However, these mutant proteins can reduce α-KG to D-2-hydroxyglutaric acid (D2HG), using NADPH as the cofactor (Figure 1B). [4,7] This new function leads to a high cellular level of D2HG, which is an inhibitor of α-KG dependent histone demethylases as well as DNA methyl hydroxylases, [8] causing a genome-wide histone/DNA hypermethylation phenotype. Recent studies showed introducing R132H IDH1 mutation recapitulated the phenotype and blocked cell differentiation of the recipient cells. [9,10] These lines of evidence have indicated that IDH mutation is a key step towards oncogenesis and a potential drug target for intervention.

There has been a significant amount of interest in discovering inhibitors of mutant IDH during the past few years. [11–16] Figure 1C shows structurally distinct inhibitors of mutant IDH. Several inhibitors have been in clinical trials against IDH mutated cancer. [17] Given the limited number of available inhibitors as well as the high potential to become potential therapeutics for these cancers, more chemotypes of inhibitors of mutant IDH are needed. Here, we report the discovery and structure activity relationships (SAR) of aromatic sulfonamides as a new class of inhibitors of IDH1(R132H). Antitumor activities of selected compounds are also reported against primary tumor cells from GBM patients bearing IDH1 R132H mutation.

MATERIALS AND METHODS

All reagents were purchased from Alfa Aesar (Ward Hill, MA) or Aldrich (Milwaukee, WI). All compounds were characterized by ^{1}H spectrum on a Varian (Palo Alto, CA) 400-MR spectrometer. The purities were determined by a Shimadzu Prominence HPLC with a Zorbax C18 or C8 column (4.6 × 250 mm) or ^{1}H (at 400 MHz) absolute spin-count quantitative NMR analysis with imidazole as an internal standard. The purities of all synthesized compounds were found to be >95%.

Chemical synthesis of compounds **1**-**20** is illustrated in Scheme 1 and described in detail below.

General Method for Reaction 1.

(i) To a solution of an aromatic amine compound (1.38 mmol) in pyridine (3 mL), an aromatic sulfonyl chloride (1.38 mmol) solution in pyridine (3 mL) was added dropwise at 0 ^{o}C under N_2. The mixture was stirred overnight. Pyridine was removed under reduced pressure. The oily residue was dissolved in ethyl acetate (20 mL), washed with water (2 × 10 mL) and brine, and concentrated. The residue was subjected to column chromatography (silica gel) to give a sulfonamide product in 66–89% yield.

General Methods for Reactions 2.

(ii) To a mixture of 4-nitro-1-naphthanol (10 mmol) in acetic acid (25 mL), Br_2 (10 mmol, 1.60 g) in acetic acid (5 mL) was added dropwise at 5–10 ^{o}C. The mixture was allowed to stir at room temperature for 20min. The solvent was removed under reduced pressure to yield 3-bromo-4-nitro-1-naphthanol as a yellow solid in 99% yield. It can be use directly for next step without further purification.

(iii) A mixture of 3-bromo-4-nitro-1-naphthanol (1 mmol), BnBr (3 mmol, 0.35 mL), and K_2CO_3 (2 mmol) in acetone (3 mL) was heated to 70 ^{o}C for 20 h in a sealed tube. The mixture was diluted with ethyl acetate (20 mL), washed with water (2 × 10 mL), dried over anhydrous Na_2SO_4 and concentrated. Hexane (1 mL) was added into the resulting residue to give benzyl protected 3-bromo-4-nitro-1-naphthanol as a yellow solid **5** in ~90% yield.

(iv) For coupling with a boronic acid: A mixture of the above product (0.67 mmol), an aromatic boronic acid (1 mmol), K_2CO_3 (2 mmol), $Pd(dppf)Cl_2$ (0.03 mmol) in dioxane (2 mL) was heated to 120 ^{o}C for 36 h in a sealed tube filled with N_2. The Suzuki coupling product was obtained with column chromatography (silica gel) in 66–81% yield. For coupling with phenol: A mixture of the above product (0.28 mmol), Phenol (0.42 mmol), CuI (0.28 mmol), N, N-dimethylglycine (0.28 mmol) Cs_2CO_3 (0.56 mmol) in dioxane (2 mL) was heated to 105 ^{o}C for 20 h in a sealed tube filled with N_2. The solvent was removed under reduced pressure, diluted with ethyl acetate and the pH was adjusted to 3–4 with HCl (aq). The product was washed with NH_4Cl, and water, and purified with column chromatograph (silica gel) in 58–76% yield.

(v) A mixture of the product from (iv) (1 mmol), 2 N HCl (0.2 mL) and 10% Pd/C (20 mg) in 5 mL CH_3OH was stirred overnight under H_2 atmosphere. The catalyst was filtered off and the filtrate was concentrated and dried under reduced pressure to yield 3-substituted 4-amino-1-naphthanol in quantitative yield.

(vi) A mixture of 4-nitro-1-naphthanol (1 mmol), paraformaldehyde (5 mmol), $MgCl_2$ (5 mmol), Et_3N (5 mmol) in CH_3CN was refluxed overnight under N_2. The solvent was removed under reduced pressure. 1 N HCl (10 mL) and ethyl acetate (20 mL) was added. The mixture was stirred for 5 min until the solid was completely dissolved. The organic phase was collected, washed with water and brine, dried over Na_2SO_4, filtered and concentrated to obtain crude 2-formyl-4-nitro-1-naphthanol, which can be used directly for the next reaction.

(vii, viii) To a solution of 2-formyl-4-nitro-1-naphthanol (1 mmol), triethyl phosphonoacetate (1.5 mmol) in 4 mL THF, NaH (2 mmol 80mg 60% in mineral oil) was added portion wise at 0 ^{o}C. After addition, the mixture was allowed to stir overnight at room temperature. Then it was diluted with ethyl acetate, neturalized with HCl(aq.), washed with water and concentrated. The residue was purified through column chromatograph (silica gel, ethyl acetate). The product was readily hydrolyzed by NaOH (2N) in MeOH/THF to give 3-(4-nitro-1-hydroxy-2-naphthyl)propionic acid.

(ix, x) To a solution of 2-formyl-4-nitro-1-naphthanol (1 mmol), tetraethyl methylenediphosphonate (1.5 mmol) in 4 mL THF, NaH (2 mmol 80mg 60% in mineral oil) was added portion wise at 0 ^{o}C. After addition, the mixture was allowed to stir overnight at room temperature. Then it was diluted with ethyl acetate, neturalized with HCl(aq.), washed with water and concentrated. The residue was purified through column chromatograph (silica gel, ethyl acetate) to give 4-nitro-2-diethylphosphonoethylenyl-1-naphthanol, which was hydrogenated and reacted with 4-bromo-phenylsulfonyl chloride using the above conditions to give diethyl ester of compound **6**. To a solution of the ester (0.5 mmol) in CH_2Cl_2 (7 mL), bromotrimethylsilane (2.5 mmol) was added dropwise at 0 ^{o}C under N_2. The mixture was allowed to stir for 36 h at room temperature under N_2. The solvent was removed under reduced pressure. MeOH (5 mL) was added to the residue and stirred for 5 min. Upon removal of the solvent, the residue was stirred with CH_2Cl_2 to give compound **6** as an off-white powder in 40% overall yield.

Compound characterization

4-bromo-N-(4-hydroxy-3-phenoxynaphthalen-1-yl)benzenesulfonamide (compound 1).—^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (d, *J* = 7.2 Hz, 1H), 7.80 (d, *J* = 7.2 Hz, 1H), 7.53–7.40 (m, 6H), 7.35 (t, *J* = 7.2 Hz, 2H), 7.19 (t, *J* = 7.2 Hz, 1H), 6.90 (m, 3H), 6.43 (s, 1H), and 6.06 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 142.3, 138.4, 138.1, 136.4, 132.3, 132.2, 130.0, 128.9, 128.7, 126.5, 126.3, 123.9, 123.1, 122.2, 122.0, 120.8, 118.5, and 117.6; MS (ESI) $[M+H]^+$ 471.3.

4-bromo-N-(3-bromo-4-hydroxynaphthalen-1-yl)benzenesulfonamide (2).—^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, *J* = 7.5 Hz, 1H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.58–7.39 (m, 7H), 6.88 (s, 1H), and 6.10 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 148.5, 138.3, 132.4, 130.4, 129.0, 128.3, 128.0, 127.8, 126.9, 124.5, 124.2, 123.1, 122.0, and 121.9; MS (ESI) $[M+H]^+$ 458.1.

N-(4-hydroxy-3-(p-tolyl)naphthalen-1-yl)-4-methylbenzenesulfonamide (3).—^{1}H NMR (400 MHz, $CDCl_3$): δ 8.45 (s, 1H), 8.20–8.14 (m, 2H), 7.98 (d, *J* = 7.2 Hz, 2H), 7.70 (t, *J* = 7.2 Hz, 1H), 7.62 (t, *J* = 7.2 Hz, 1H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.40 (d, *J* = 7.2 Hz, 2H), 7.30–7.20 (m, 3H), 2.48 (s, 3H), and 2.40 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 163.2, 146.3, 144.1, 140.9, 138.2, 133.5, 133.4, 133.1, 132.3, 131.8, 130.8, 129.9, 129.6, 129.3, 128.3, 127.4, 127.3, 126.1, 21.7, and 21.5; MS (ESI) $[M+H]^+$ 404.5.

4-bromo-N-(1-hydroxy-[2,2'-binaphthalen]-4-yl)benzenesulfonamide (4).—^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (d, *J* = 7.2 Hz, 1H), 8.00 (d, *J* = 7.2 Hz, 1H), 7.97–7.80

(m, 4H), 7.62–7.39 (m, 10H), 7.09 (s, 1H), 6.60 (s, 1H), and 6.10 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 150.9, 146.2, 144.1, 140.7, 138.7, 135.2, 134.2, 133.5, 133.4, 133.1, 132.3, 131.9, 130.8, 129.9, 129.6, 129.3, 128.3, 127.4, 127.3, 126.3, 124.6, 126.1, 119.0, and 115.5; MS (ESI) $[M+H]^+$ 505.4.

3-(4-((4-bromophenyl)sulfonamido)-1-hydroxynaphthalen-2-yl)propanoic acid (5).—^{1}H NMR (400 MHz, DMSO-d_6): δ 9.95 (s, 1H), 9.36 (br, 1H), 8.14 (d, J = 7.2 Hz, 1H), 7.80 (d, J = 7.2 Hz, 1H), 7.70 (t, J = 7.2 Hz, 2H), 7.50 (d, J = 7.2 Hz, 2H), 7.40 (d, J = 7.2 Hz, 2H), 7.38 (d, J = 7.2 Hz, 2H), 6.78 (s, 1H), 2.84 (t, J = 4.8 Hz, 2H), and 2.45 (t, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 176.5, 150.3, 143.3, 138.7, 133.9, 131.0, 129.7, 129.5, 127.9, 126.7, 125.7, 124.0, 123.7, 121.8, 120.1, 34.1 and 24.6; MS (ESI) $[M+H]^+$ 451.3.

(2-(4-((4-bromophenyl)sulfonamido)-1-hydroxynaphthalen-2-yl)ethyl)phosphonic acid (6).—^{1}H NMR (400 MHz, $CDCl_3$): δ 9.63 (s, 1H), 8.12 (d, J = 7.2 Hz, 1H), 7.90 (d, J = 7.2 Hz, 1H), 7.42 (t, J = 7.2 Hz, 2H), 7.40–7.30 (m, 2H), 7.22 (d, J = 7.2 Hz, 2H), 6.60 (br, 1H), 2.78–2.70 (m, 2H), and 1.63–1.58 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 143.3, 137.9, 136.5, 132.2, 129.6, 128.9, 128.3, 127.7, 125.4, 123.7, 122.7, 121.8, 120.1, 107.5, 29.6 (d, $J_{C\text{-}P}$ = 98 Hz), and 27.9 (d, $J_{C\text{-}P}$ = 33 Hz); ^{31}P NMR (162 MHz, DMSO-d_6): 26.0; MS (ESI) $[M-H]^-$ 485.3.

3-(1-hydroxy-4-((4-methoxyphenyl)sulfonamido)naphthalen-2-yl)propanoic acid (7).—^{1}H NMR (400 MHz, $CDCl_3$): δ 11.0 (br, 1H), 8.42 (br, 1H), 8.27 (d, J = 8.3 Hz, 1H), 7.69 (d, J = 8.3 Hz, 1H), 7.59 (d, J = 8.9 Hz, 2H), 7.41 (t, J = 7.3 Hz, 1H), 7.34 (t, J = 7.3 Hz, 1H), 6.98 (s, 1H), 6.79 (d, J = 8.9 Hz, 2H), 6.71 (s, 1H), 3.78 (s, 3H), 2.91 (t, J = 5.5 Hz, 2H), and 2.69 (t, J = 5.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.2, 163.1, 149.8, 131.0, 130.3, 129.7, 129.5, 127.9, 126.7, 125.7, 124.0, 123.2, 121.8, 120.2, 114.0, 55.7, 35.1 and 24.3; MS (ESI) $[M+H]^+$ 402.4.

N-(3-bromo-4-hydroxynaphthalen-1-yl)-4-fluorobenzenesulfonamide (8).—^{1}H NMR (400 MHz, $CDCl_3$): δ 10.5 (br, 1H), 8.15 (d, J = 7.0 Hz, 1H), 7.80 (d, J = 7.0 Hz, 1H), 7.64 (d, J = 7.0 Hz, 2H), 7.48 (t, J = 8.0 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 7.30 (t, J = 8.0 Hz, 2H), and 7.03 (d, J = 8.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 165.4 (d, $^1J_{F\text{-}C}$ = 254 Hz), 148.4, 135.2, 130.4, 130.2 (d, $^3J_{F\text{-}C}$ = 9.7 Hz), 128.0, 127.8, 126.8, 124.4 (d, $^2J_{F\text{-}C}$ = 17.6 Hz), 123.0, 122.0, 116.5, 116.3, and 102.6; MS (ESI) $[M+H]^+$ 397.2.

N-(3-bromo-4-hydroxynaphthalen-1-yl)-4-methylbenzenesulfonamide (9).—^{1}H NMR (400 MHz, $CDCl_3$): δ 10.0 (s, 1H), 9.95 (s, 1H), 8.15 (d, J = 7.5 Hz, 1H), 7.85 (d, J = 7.5 Hz, 1H), 7.50–7.39 (m, 4H), 7.25 (d, J = 7.0 Hz, 2H), 7.01 (s, 1H), and 2.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 148.1, 144.1, 136.2, 130.5, 129.7, 127.63, 127.60, 127.54, 126.7, 124.8, 124.5, 122.9, 122.3, 102.6, and 21.7; MS (ESI) $[M+H]^+$ 393.3.

N-(3-bromo-4-hydroxynaphthalen-1-yl)-[1,1'-biphenyl]-4-sulfonamide (10).—^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, J = 7.5 Hz, 1H), 7.76 (d, J = 7.5 Hz, 2H), 7.67 (d, J = 7.5 Hz, 1H), 7.60–7.39 (m, 10H), 6.47 (s, 1H), and 5.59 (s, 1H); ^{13}C NMR (100 MHz,

$CDCl_3$): 148.2, 142.1, 139.1, 134.4, 129.0, 128.5, 127.86, 127.84, 127.57, 127.53, 127.39, 127.27, 126.6, 122.8, 122.0, 118.6, 113.9, and 110.0; MS (ESI) $[M+H]^+$ 455.3.

N-(3-bromo-4-hydroxynaphthalen-1-yl)naphthalene-2-sulfonamide (11).—^1H NMR (400 MHz, $CDCl_3$): δ 10.15 (s, 1H), 10.0 (s, 1H), 8.20 (s, 1H), 8.13–7.97 (m, 4H), 7.90 (d, *J* = 7.5 Hz, 1H), 7.75 (d, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 1H), 7.58 (t, *J* = 7.5 Hz, 1H), 7.42 (t, *J* = 7.5 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), and 7.01 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 148.4, 136.2, 135.0, 132.1, 130.6, 129.5, 129.4, 129.08, 129.06, 128.0, 127.8, 127.7, 126.8, 125.3, 124.6, 122.9, 122.5, 122.2, 122.1, and 102.6; MS (ESI) $[M+H]^+$ 429.3.

N-(4-hydroxynaphthalen-1-yl)-4-methylbenzenesulfonamide (12).—^1H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 9.82 (s, 1H), 8.08 (d, *J* = 7.6 Hz, 1H), 7.81 (d, *J* = 7.6, 3H), 7.61–7.52 (m, 2H), 7.45 (d, *J* = 7.6 Hz, 2H), 7.14 (d, *J* = 7.6 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), and 2.41 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 146.4, 142.3, 136.2, 132.0, 130.7, 128.7, 128.1, 127.6, 127.5, 126.9, 124.6, 123.1, 122.1, 118.9, and 21.6; MS (ESI) $[M+H]^+$ 314.4.

N-(4-hydroxynaphthalen-1-yl)benzenesulfonamide (13).—^1H NMR (400 MHz, DMSO-d_6) δ 10.28 (s, 1H), 9.82 (s, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 7.85 (d, *J* = 7.8, 1H), 7.63 (d, *J* = 7.2 Hz, 2H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.50–7.46 (m, 2H), 7.39–7.37 (m, 2H), 6.86 (d, *J* = 7.6 Hz, 1H), and 6.71 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) 151.2, 139.1, 131.3, 130.4, 127.8, 125.6, 124.9, 124.4, 123.7, 123.6, 122.05, 122.01, 120.9, and 106.1; MS (ESI) $[M+H]^+$ 300.3.

4-bromo-N-(4-hydroxy-3-phenoxyphenyl)benzenesulfonamide (14).—^1H NMR (400 MHz, $CDCl_3$): δ 7.56–7.48 (m, 4H), 7.44–7.30 (m, 2H) , 7.19–7.14 (m, 1H), 6.92–6.84 (m, 2H), 6.76–6.68 (m, 3H), 6.53–6.50 (m, 1H), and 5.71 (br, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 155.9, 146.1, 144.0, 137.7, 133.1, 132.4, 130.2, 129.0, 124.4, 120.8, 120.0, 118.4, 116.9, and 114.6; MS (ESI) $[M+H]^+$ 421.3.

4-bromo-N-(4-methoxy-3-phenoxyphenyl)benzenesulfonamide (15).—^1H NMR (400 MHz, $CDCl_3$): δ 7.55–7.50 (m, 4H), 7.31–7.26 (m, 2H), 7.08 (t, *J* = 7.6 Hz, 1H), 6.87 (d, *J* = 1.2 Hz, 2H), 6.80 (dd, *J* = 7.6, 1.2 Hz, 2H), 6.60 (s, 1H), 6.54 (s, 1H), and 3.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 157.4, 146.7, 141.0, 138.9, 138.7, 133.1, 132.4, 130.2, 129.1, 124.6, 120.8, 120.0, 116.4, 111.7, and 56.8; MS (ESI) $[M+H]^+$ 435.3.

4-bromo-N-(4-methoxyphenyl)benzenesulfonamide (16).—^1H NMR (400 MHz, $CDCl_3$): δ 9.97 (s, 1H), 8.16 (d, *J* = 7.2 Hz, 2H), 7.63 (d, *J* = 7.2 Hz, 2H), 6.94 (d, *J* = 1.2 Hz, 2H), 6.81 (dd, *J* = 7.6, 1.2 Hz, 2H), and 3.83 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 153.4, 138.7, 132.9, 130.7, 129.5, 126.3, 124.5, 115.4, and 55.8; MS (ESI) $[M+H]^+$ 343.2.

4-bromo-N-(4-hydroxyphenyl)benzenesulfonamide (17).—^1H NMR (400 MHz, $CDCl_3$): δ 9.97 (s, 1H), 9.90 (s, 1H), 8.18 (d, *J* = 7.2 Hz, 2H), 7.69 (d, *J* = 7.6 Hz, 2H), 6.81 (d, *J* = 7.2 Hz, 2H), and 6.60 (d, *J* = 7.6, Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): 148.4, 138.7, 131.3, 130.3, 129.1, 126.3, 124.5, and 117.4; MS (ESI) $[M+H]^+$ 329.2.

4-bromo-N-(3-bromophenyl)benzenesulfonamide (18).—^{1}H NMR (400 MHz, $CDCl_3$): δ 10.02 (s, 1H), 9.96 (s, 1H), 8.18 (d, J = 7.2 Hz, 2H), 7.61 (d, J = 7.2 Hz, 2H), 7.01–6.70 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): 140.4, 138.7, 131.7, 130.3, 129.1, 126.3, 124.5, 121.6, 118.4, and 115.3; MS (ESI) $[M+H]^+$ 392.1.

4-bromo-N-phenylbenzenesulfonamide (19).—^{1}H NMR (400 MHz, $CDCl_3$): δ 10.01 (s, 1H), 8.07 (d, J = 7.2 Hz, 2H), 7.62 (d, J = 7.2 Hz, 2H), 7.20–7.15 (m, 2H), and 6.85–6.80 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 137.7, 136.0, 132.3, 129.4, 128.7, 128.1, 125.6, and 121.7; MS (ESI) $[M+H]^+$ 313.2.

4-bromo-N-(3-chloro-4-fluorophenyl)benzenesulfonamide (20).—^{1}H NMR (400 MHz, $CDCl_3$): δ 9.95 (s, 1H), 8.06 (d, J = 7.2 Hz, 2H), 7.66 (d, J = 7.2 Hz, 2H), and 7.30–6.60 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 151.6 (d, $^1J_{F\text{-}C}$ = 237 Hz), 143.2, 141.2, 132.0, 127.8, 125.7, 121.0, 116.8, 116.4, and 114.3; MS (ESI) $[M+H]^+$ 365.6.

Enzyme Inhibition.—Inhibitory activities of compounds were tested against mutant and wild-type IDH1 using our previous methods. [13]

Steady-state kinetic study.—Steady-state kinetic inhibition experiment was performed by determining initial velocities of reactions catalyzed by IDH1(R132H) with varying the concentrations of compound **9**, α-KG and NADPH. Data were imported into SigmaPlot (version 13, Systat Software, Inc.) and fitted to competitive, noncompetitive and uncompetitive inhibition models. The best kinetic models were determined by the highest R^2 and lowest AICc values. Lineweaver-Burk or Michaelis-Menten plots were generated by Sigmaplot.

Inhibition of proliferation of glioma stem-like cells.—Two glioma cell lines BT142 and BXD-3752 were cultured as neurospheres in serum-free cell growth media at 37 °C in a 5% CO_2 atmosphere with 100% humidity as described previously in Ref. 13. In brief, 2000 cells/well were added into 96-well plates and treated with increasing concentrations of a compound for 14 days. Cell viability was determined by Cell Counting Kit-8 (Dojindo Molecular Technologies, Rockville, MD) according to the manufacturer's instructions.

RESULTS and DISCUSSION

Biochemical assay and inhibitor discovery.

A biochemical assay was used to screen for new inhibitors of IDH1(R132H), which was expressed in *E. coli* and purified using our previous methods. [12–14] The assay is based on an initial linear consumption of NADPH, which has a maximal UV absorbance at 340 nm. Thus, an initial velocity of the enzyme reaction can be determined to be the decreasing rate of [NADPH] monitored by UV. We performed compound screening at 50 μM followed by IC_{50} (concentration at which a compound can inhibit the enzyme activity by 50%) and K_i (inhibition constant) determination for active hits, compound **1** (Figure 2) was found to be a novel inhibitor of IDH1(R132H) with a K_i value of 8.2 μM.

Chemical synthesis.

Methods for synthesizing these sulfonamide compounds are illustrated in Scheme 1. All sulfonamide compounds were synthesized by the reaction of a commercially available sulfonyl chloride and a substituted naphthyl or phenyl amine (Reaction 1). Except for a few that are commercially available, these amines were synthesized according to Reactions 2. 4-Nitro-1-naphthol or -phenol was treated with Br_2 to add a 2-bromo group and 1-OH of the product was protected with a benzyl group. An ensuing metal-catalyzed reaction can substitute the 2-Br with the aromatic group (found in compounds **1**, **3**, **4**, **14** and **15**). The product was hydrogenated to give the desired 2-substituted naphthyl or phenyl amine for Reaction 1. 4-Nitro-1-naphthol was formylated at 2-position by treatment with paraformaldehyde in the presence of $MgCl_2$ and triethylamine. The 2-formyl group can be converted to side chains in compounds **5** - **7**, using methods shown in Reactions 2.

Structure activity relationship (SAR) studies.

We next performed structure activity relationship (SAR) studies based on the structure of **1**, in an effort to find more potent inhibitors. Compounds **2** - **7** (Figure 2) were synthesized to see the effects of 2-substituents. Compound **2** with a 2-Br exhibited ~2-fold increased inhibitory activity (K_i = 4.1 μM). However, compounds **3** and **4** having a 4-tolyl and 2-naphthyl group at this position, respectively, were inactive, showing a direct aromatic substituent is disfavored. Compound **5** bearing a carboxylethyl group showed reduced activity with K_i of 17.5 μM, as compared with compounds **1** and **2**. However, compound **6** with a 2-phosphono-ethyl substituent was found to have a considerably improved activity of 2.4 μM. Compound **7** with a 4-methoxybenzenesulfonyl moiety showed increased activity (K_i = 10.2 μM), as compared to **5**. Because highly polar and negatively charged phosphonic compounds are generally not cell permeable, 2-Br was used for optimization of other positions.

2-Bromo compounds **8** - **11** with different aromatic sulfonyl groups were synthesized and tested for their activities inhibiting IDH1(R132H). As shown in Figure 2, compound **8** with a small, more electron-withdrawing 4-F substituted benzenesulfonyl was found to be significantly less active (K_i ~25 μM) than compound **2** with a 4-Br. On the other hand, compound **9** with a 4-methylbenzenesulfonyl is a strong inhibitor with a K_i value of 1.2 μM, ~4× more active than **2**. Compounds **10** and **11** bearing a 4-biphenyl and 2-naphthyl sulfonyl group showed the most potent inhibitory activity with K_i values of 0.6 and 0.8 μM. These results suggest that electron-donating and/or bulky aromatic groups are more favored at this position.

Compound **12** (K_i = 6.8 μM, Figure 2) is ~6-fold less active than compound **9**, showing the 2-Br group in **9** is significantly more favorable than the 2-H. In addition, as compared to **12**, reduced activity of compound **13** (K_i = 11.1 μM) indicates that the 4-methyl group in the benzenesulfonyl moiety is a favorable substituent.

A series of phenylamine sulfonamide compounds **14 – 20** shown in Figure 3 were synthesized to further probe the SARs of this class of compounds. Devoid of activity for compound **14** clearly indicates the importance of the corresponding naphthyl ring in

compound **1**, in which the additional aromatic ring possibly provides favorable hydrophobic interactions with the protein. 1-Methoxy group in compound **15** as well as **16** does not seem to render an improved potency. However, compound **17** having a 1-OH showed enhanced activity (K_i = 7.3 μM), which is considerably more active than **16** with a 1-OMe. This shows the hydroxy group contributes significantly to the inhibition. Nevertheless, compound 18, which does not carry a -OH but has a 2-Br group, exhibited a comparable activity (K_i = 9.0 μM) to that of compound **17**. Therefore, it can be inferred from these results that the 2-Br group also plays an important role in enzyme activity inhibition. This point can be further supported by the activities of compounds **19** and **20**. Without the corresponding -Br group, compound **19** is inactive. Compound **20** having a smaller -Cl at this position is a weaker inhibitor of IDH1(R132H) (K_i = 16.1 μM), although it is possible that the -F group also contributes to the inhibition.

Enzyme kinetics studies

To find mode of action of this series of compounds, we did steady-state enzyme kinetic studies for compound **9**. Initial velocities of IDH1(R132H) were determined with increasing concentrations of **9**, the substrate α-KG and cofactor NADPH. The data were fitted and analyzed to competitive, uncompetitive and noncompetitive kinetics models by the program SigmaPlot. The mode of action for compound **9** to inhibit the enzyme can be determined by the best fitting, as judged by the R^2 and AICc values, using a Lineweaver-Burk or Michaelis-Menten plot. With this method, compound **9** was found to be a competitive inhibitor against the substrate α-KG, as shown in Figure 4A. It was also found to exhibit a noncompetitive mode of action against the cofactor NADPH (Figure 4B).

Evaluation of anti-glioma activity

Finally, we tested antitumor activities of potent inhibitors **9** and **10** against proliferation of BT142 glioma cells harboring R132H IDH1 mutation (Figure 5). In the meantime, activity of these compounds against BXD-3752 glioma cells without an IDH1 mutation was also evaluated. Inactive compound **3** was included in these assays as a control to find possible off-target toxicity of these sulfonamide compounds. These glioma cells were derived from patient samples and cultured in serum-free media to form colony-like “neurospheres”. [13,14] Stem-like cancer cells were found to be enriched in these neurospheres, which have the ability to self-renew and differentiate. Inhibition of these stem-like tumor cells is of importance, given roles of these cells in drug resistance, relapse and metastasis. As shown in Figure 5A, compounds **9** and **10** at 5 μM strongly inhibited the growth of BT142 glioma cells with R132H IDH1 mutation, while these two compounds did not show activity against BXD-3752 cells. In addition, inactive compound **3** did not exhibit activity against proliferation of both glioma cells. These results show that potent inhibitors of IDH1(R132H) have selective activity against glioma cells containing the IDH1 mutation.

CONCLUSION

In summary, a series of aromatic sulfonamide compounds have been found to be novel, potent inhibitors of R132H mutant IDH1 with K_i values as low as 0.6 μM. This work is of interest because this specific mutation has been found to be a drug target in ~75% low-grade

gliomas as well as secondary GBM. Several structure activity relationships have been concluded, which might be useful to guide further inhibitor optimization. For the aromatic amine moiety, 1-hydroxy and 2-bromo groups have been found to be critical to the inhibition of IDH1(R132H). 1-hydroxy-naphthylamine is superior to the corresponding phenylamine, presumably due to enhance hydrophobic interactions. For the benzenesulfonyl moiety, a bulkier (such as phenyl in **10**) and/or an electron-releasing group (such as methyl in **9**) at the 4-position are favorable. Enzyme kinetics studies showed that compound **9** is a competitive inhibitor of IDH1(R132H) against the substrate α-KG and a non-competitive inhibitor against the cofactor NADPH. In addition, potent sulfonamide inhibitors of IDH1(R132H) are highly selective, showing no or negligible inhibitory activity against WT or R132C IDH1 (Table 1). Potent inhibitors **9** and **10** exhibited strong activity against proliferation of BT142 glioma cells with IDH1 R132H mutation, while these compounds did not significantly affect growth of BXD-3752 cells without IDH1 mutation. These results suggest further characterization and optimization of these compounds are warranted with a goal to find a clinically useful drug targeting IDH1 mutated gliomas.

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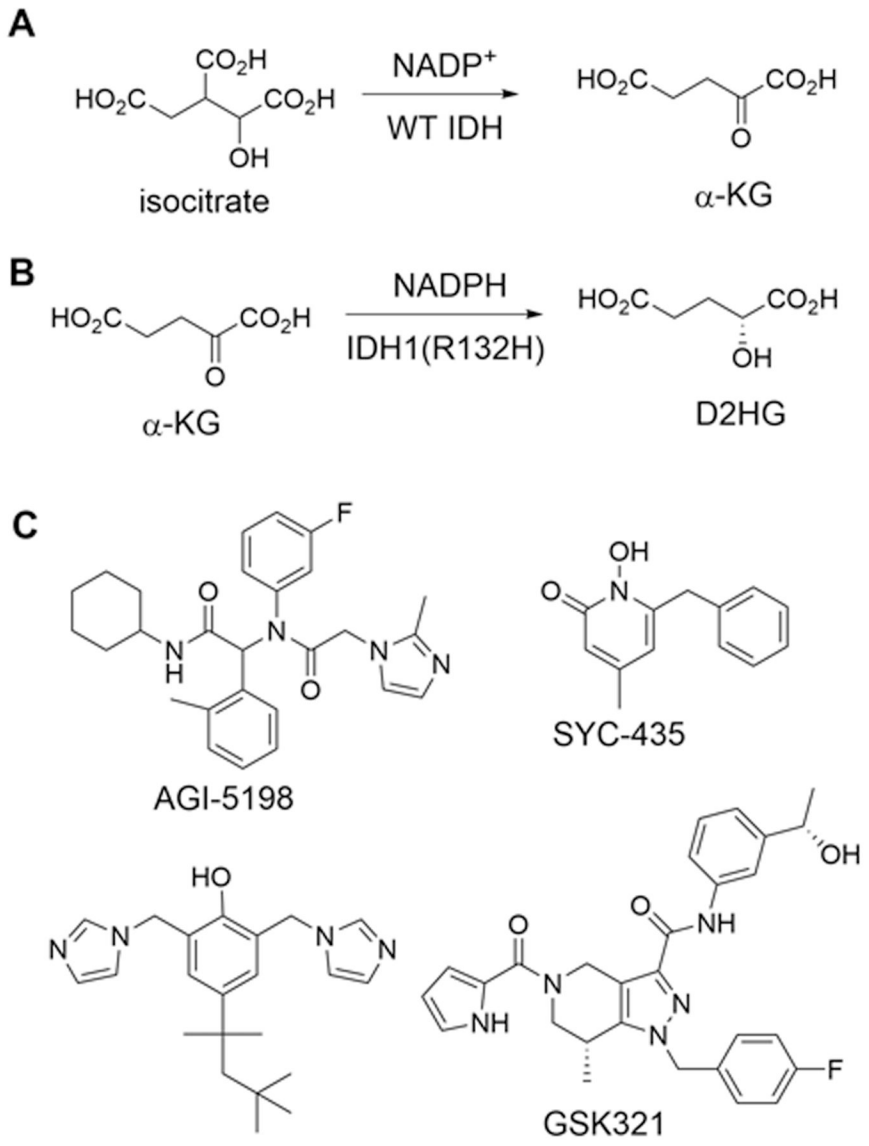


Figure 1.

Reactions catalyzed by (**A**) WT IDH1 and (**B**) IDH1(R132H); (**C**) Representative inhibitors of mutant IDH1.

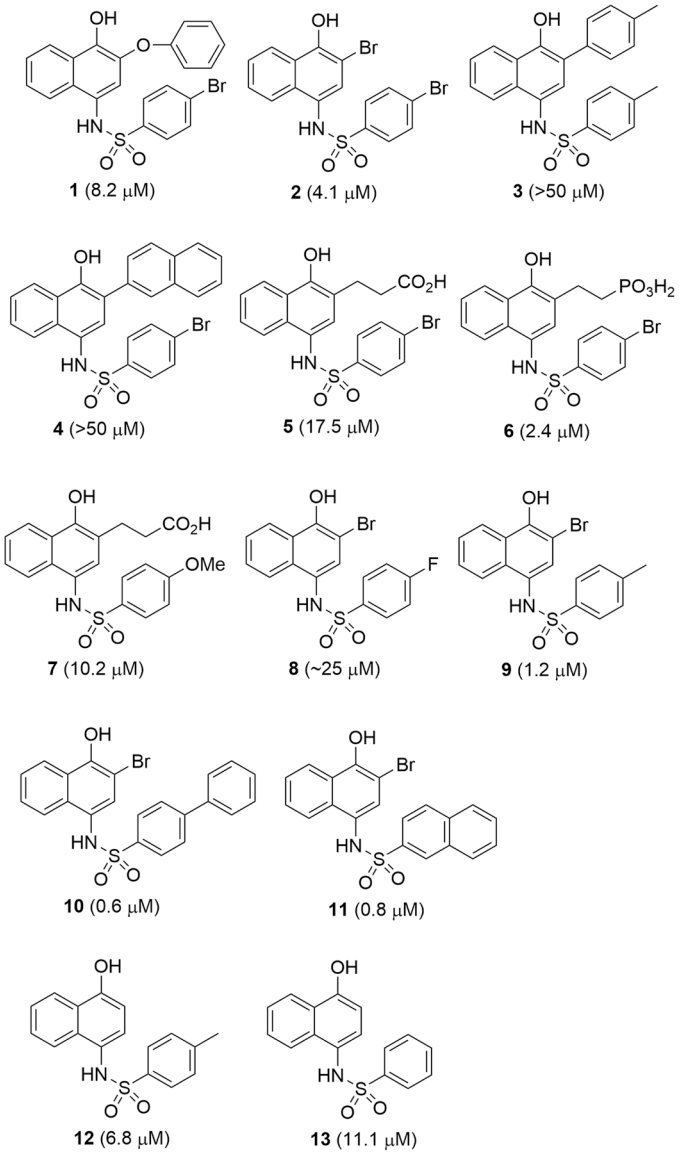


Figure 2.
Structures and inhibitory activities of compounds **1-13**.

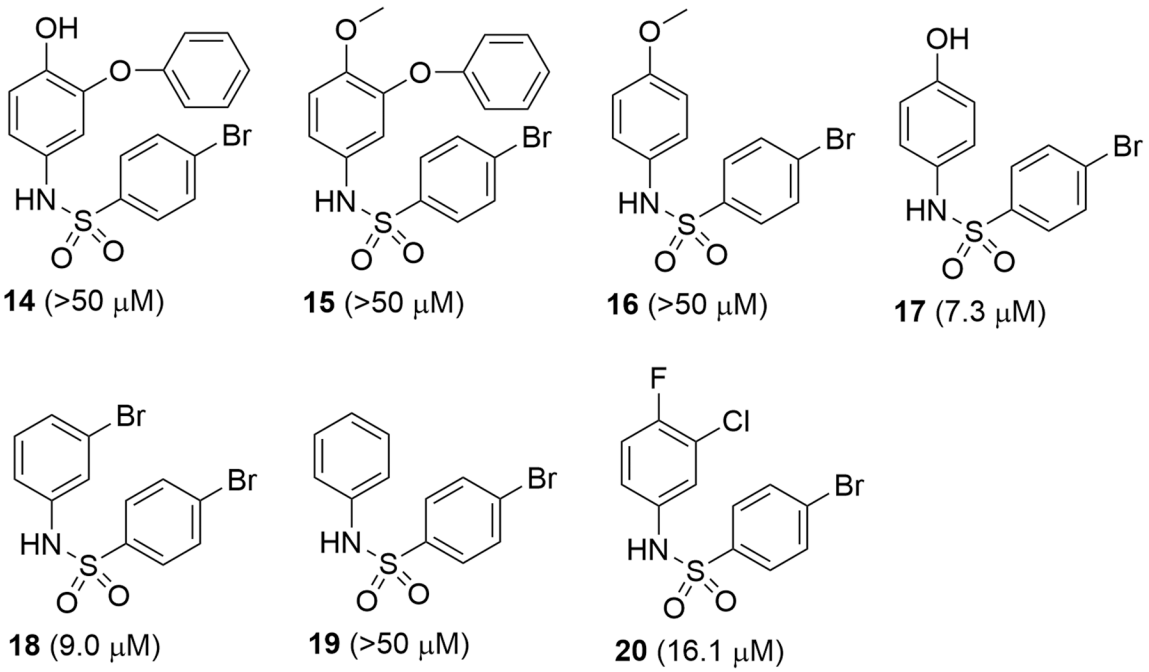


Figure 3.
Structures and activities of compounds **14**-**20**.

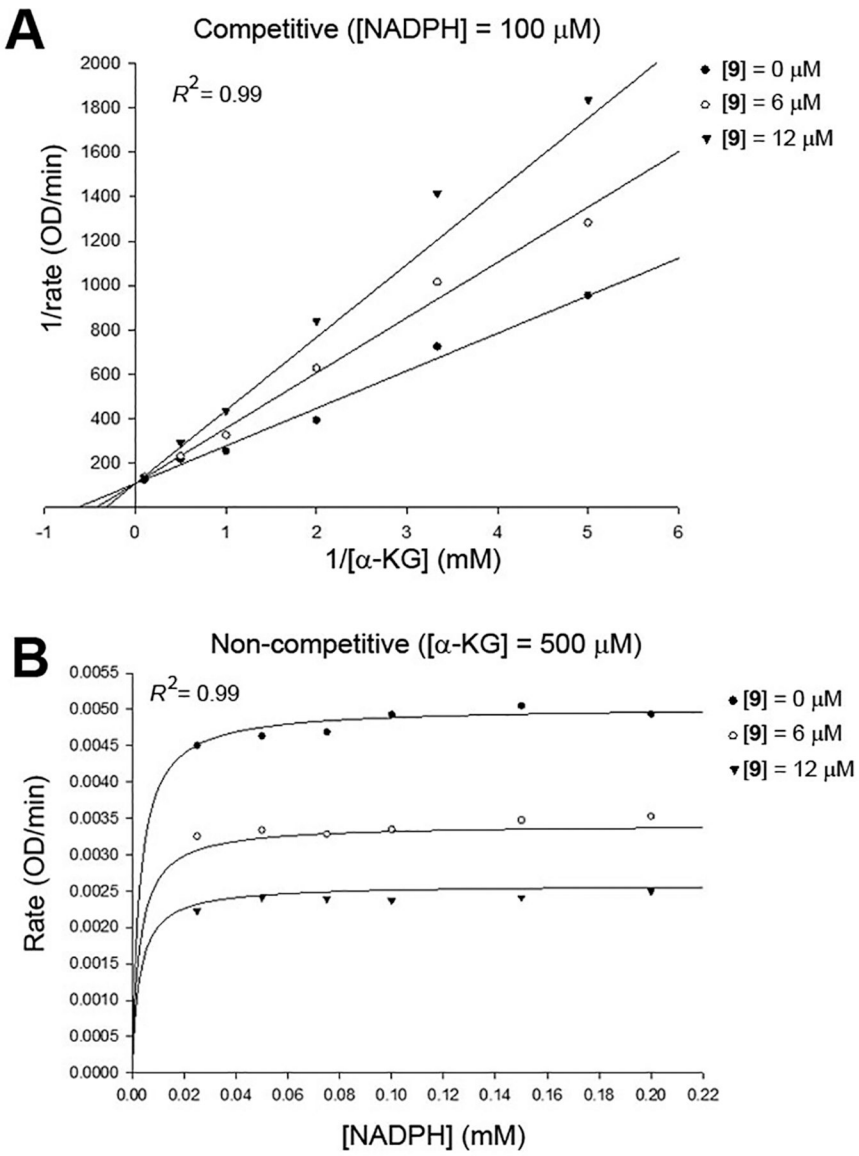


Figure 4.

The best fitting models for enzyme kinetic studies of compound **9**. (**A**) Competitive inhibition model with variable concentrations of α-KG using a Lineweaver-Burk plot; and (**B**) Noncompetitive inhibition model with variable concentrations of NADPH using a Michaelis-Menten plot.

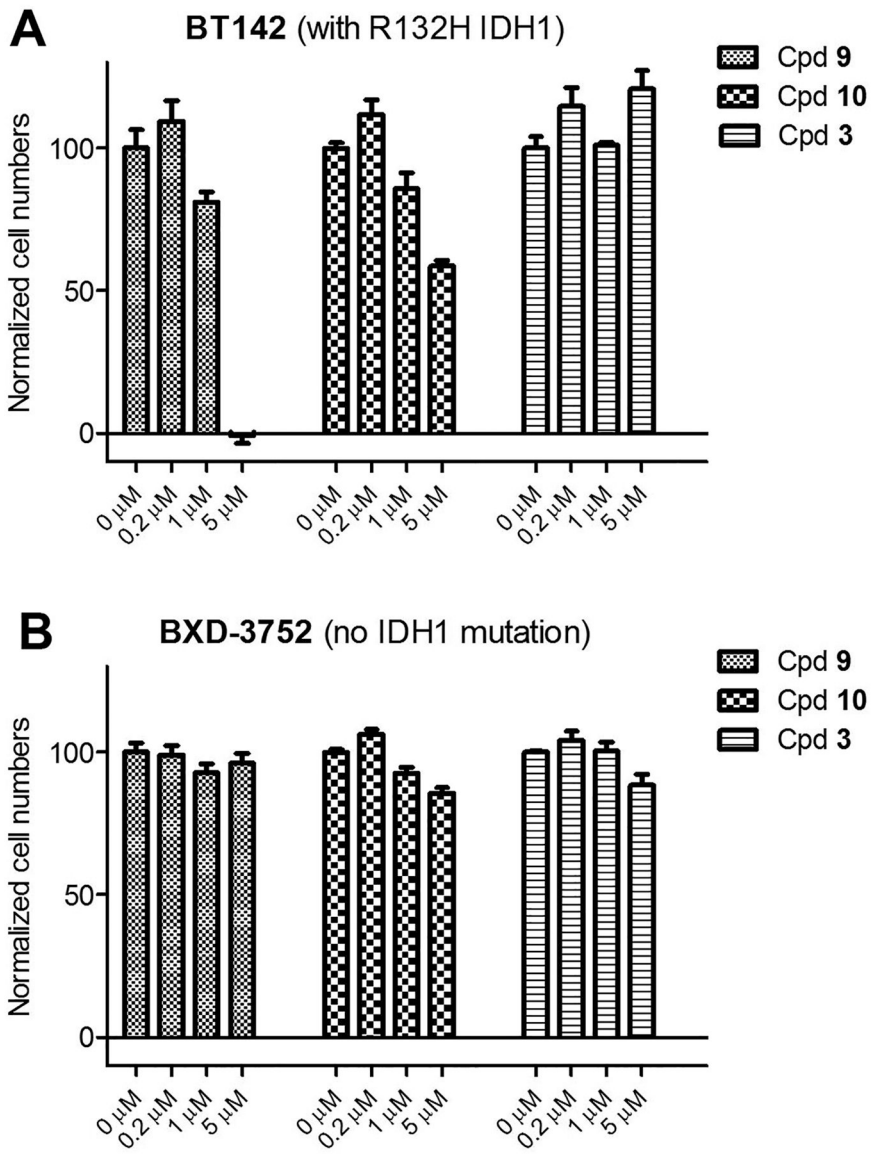
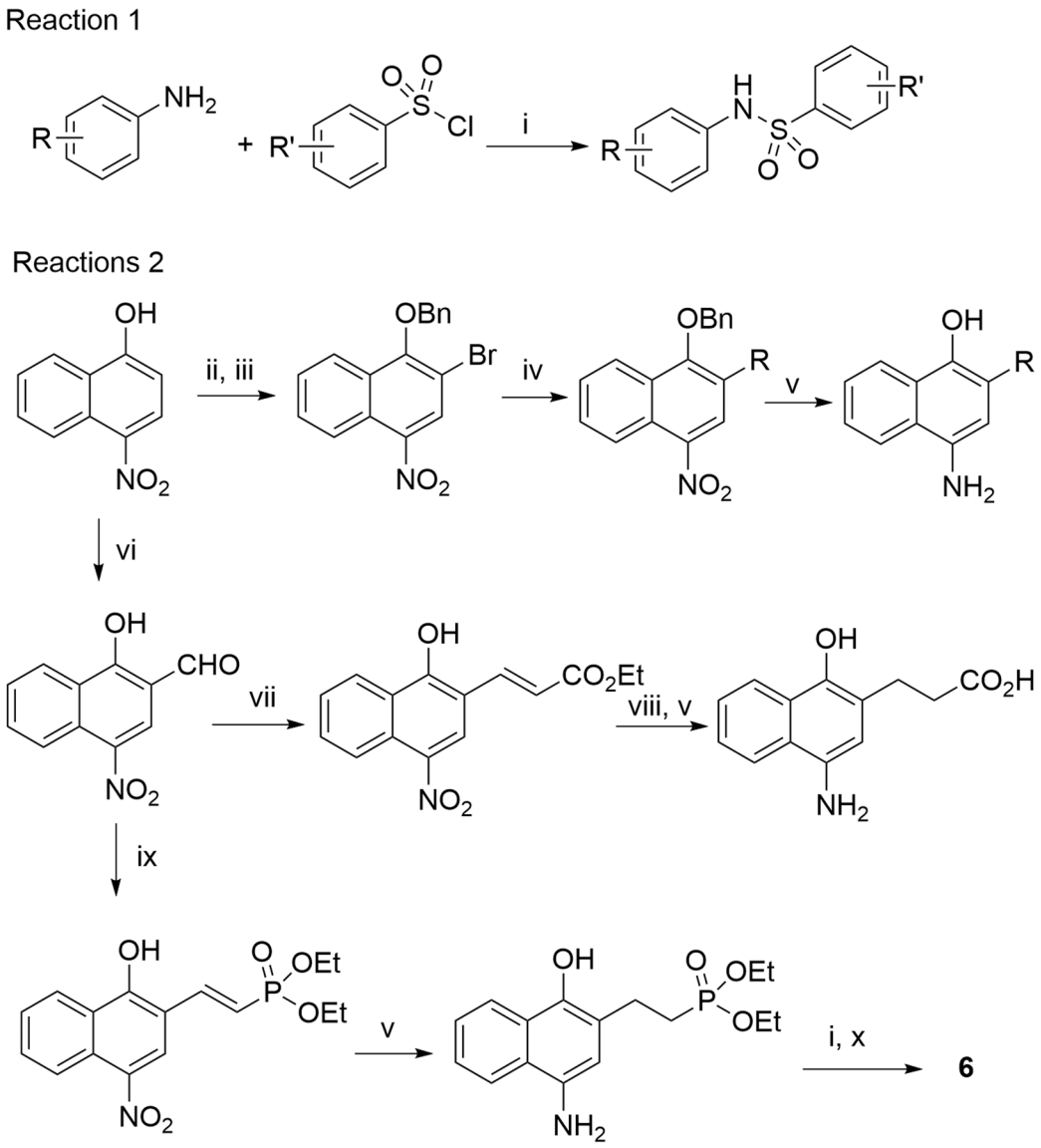


Figure 5.
Activity of compounds **9**, **10** and **3** against BT142 and BXD-3752 glioma cells.



Scheme 1.

Synthetic methods.[a]

[a]*Reagents and conditions:* (i) pyridine; (ii) Br_2, AcOH; (iii) BnBr, K_2CO_3, acetone, reflux; (iv) for synthesizing **3** and **4**, $RB(OH)_2$, $Pd(dppf)Cl_2$, K_2CO_3, dioxane, reflux; or for synthesizing **1**, **14** and **15**, phenol, CuI, N, N-dimethylglycine, Cs_2CO_3, dioxane, reflux; (v) 10% Pd/C, H_2; (vi) paraformaldehyde, $MgCl_2$, NEt_3, CH_3CN, reflux; (vii) triethyl phosphonoacetate, NaH, THF; (viii) NaOH, MeOH; (ix) tetraethyl methylenediphosphonate, NaH, THF; (x) bromotrimethylsilane, CH_2Cl_2.

Table 1.

Activity (K_i in μM) against WT and mutant IDH1.

Cpd	R132H	R132C	WT
2	4.1	>50	>50
9	1.2	>50	>50
10	0.6	36	>50
11	0.8	>50	>50