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Isosteres of ester derived glucose uptake inhibitors

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ABSTRACT

Glucose transporters (GLUTs) facilitate glucose uptake and are overexpressed in most cancer cells. Inhibition of glucose transport has been shown to be an effective method to slow the growth of cancer cells both in vitro and in vivo. We have previously reported on the anticancer activity of an ester derived glucose uptake inhibitor. Due to the hydrolytic instability of the ester linkage we have prepared a series of isosteres of the ester moiety. Of all of the isosteres prepared, the amine linkage showed the most promise. Several additional analogues of the amine-linked compounds were also prepared to improve the overall activity.

An estimated 1.8 million new cancer cases are expected to be diagnosed in 2020 in US alone, and this will result in an estimated 606,520 deaths in the same year. 1 out of every 2 American males and 1 in every 3 females is expected to be diagnosed with cancer once in their lifetimes. The overall five-year survival rate for all types of cancers has increased from 3% in 1970s to 18% today.[1] Cancers tend to favor glycolysis over oxidative phosphorylation for biomass synthesis and ATP production. Cancer cells proliferate faster than normal human cells and outpace the diffusion limits of local blood supply. Hypoxic conditions in human tumors favor aerobic glycolysis over oxidative phosphorylation.[2] Glucose based metabolism in cancer can be targeted in several ways such as by inhibiting glycolytic enzymes or by inhibiting glucose uptake itself.[3,4] Glucose transporters (GLUTs) are proteins that facilitate uptake of glucose inside cancer cells. GLUT1-4 are found to be the most important in tumor growth, survival and metastasis. However, GLUT1 is the most upregulated transporter in a wide variety of human cancer cells.[5] Human tumors are addicted to glucose and glucose deprivation is toxic to cancer cells. Inhibiting GLUT1 and other GLUTs could be an efficient way of preventing glucose from entering the cell and undergoing glycolysis, and eventually leading to cell death by starvation. There are several natural and synthetic small organic compounds that have shown potency to inhibit GLUT-mediated glucose transport.[4] We have previously reported on a class of phenolic esters (e.g. **1 (WZB117)**) that have shown high GLUT1 inhibitory activity.[6–8] Compound **1** reduces glucose-uptake with an IC_{50} of 9.6 ± 1.0 μM and cell-growth with an IC_{50} value of 25.7 ± 6.8 μM in H1299 cells. Compound **1** has been found to suppress cancer cell growth in vitro as well as in vivo. Human A549 lung tumors were xenografted onto male nude mice and the mice were treated daily by IP injection of compound **1** at a dose of 10 mg/kg of body weight for 10 weeks. A 70% reduction in tumor volume was observed after treatment.[8]

Since our initial reports several additional GLUT inhibitors have been reported.[9,10] Some of these new GLUT inhibitors include the pyrazole BAY-876,[11,12] analogs of the macrocyclic peptide rapafucin,[13] and a series of salicylketoximes.[14,15] In addition, the use of computational methods for the discovery of GLUT inhibitors have also been reported.[16,17]

Despite the excellent activity observed with **1**, this compound could be problematic because it can be readily hydrolyzed into compounds **2** and **3** (Scheme 1), neither of which are biologically active.[6] Only about 25% of **1** remained undegraded after it was introduced in human serum for 1 h.[9]

We report herein the synthesis and evaluation of several ester isosteres that were prepared to address the poor stability of **1**. Some of the more stable isosteres of esters are amides, sulfoxides, and sulfones, and other nontraditional isosteres include amines, ethers, and sulfides.[18–21] Figure 1 shows a general structure of the compounds that were chosen to prepare in order to determine both stability issues and functional group tolerance.

The desired amides and corresponding amines were prepared as outlined in Scheme 2. Commercially available analogs of 3-hydroxybenzoic acid (**5**) were protected as either the benzyl ether or acetate

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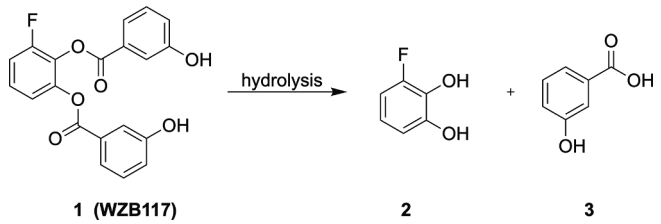


Scheme 1. Hydrolysis of **1** (**WZB117**).

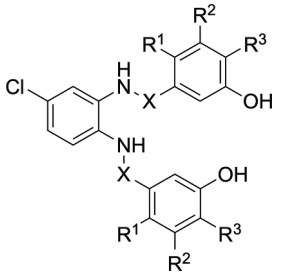
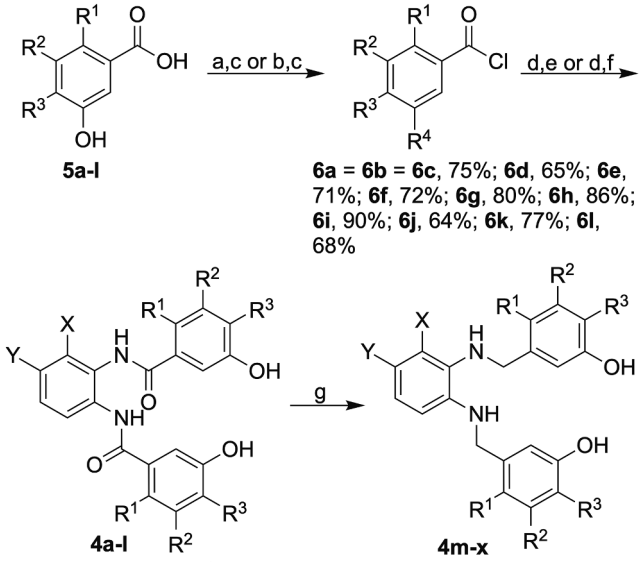


Fig. 1. Planned isosteres of diester **1**.

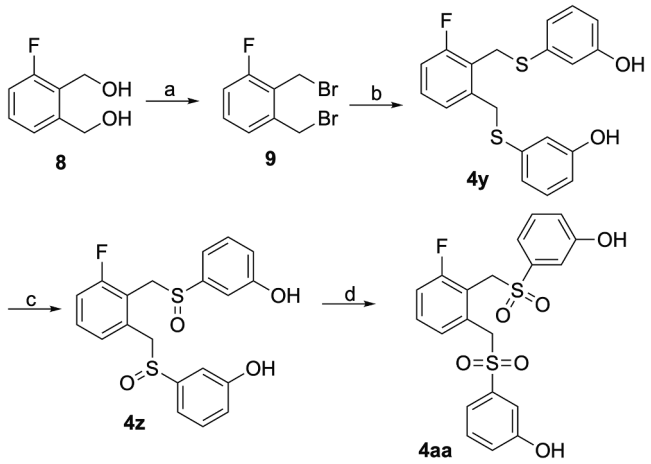


	X	Y	R^1	R^2	R^3	yield
4a	F	H	H	H	H	35%
4b	H	F	H	H	H	83%
4c	H	Cl	H	H	H	86%
4d	H	Cl	H	H	Cl	28%
4e	H	Cl	H	Cl	H	48%
4f	H	Cl	Cl	H	H	28%
4g	H	Cl	H	H	F	59%
4h	H	Cl	H	H	Br	62%
4i	H	Cl	H	H	OCH_3	42%
4j	H	Cl	H	H	CH_3	43%
4k	H	Cl	H	CH_3	H	35%
4l	H	Cl	CH_3	H	H	69%

	X	Y	R^1	R^2	R^3	yield
4m	F	H	H	H	H	53%
4n	H	F	H	H	H	66%
4o	H	Cl	H	H	H	74%
4p	H	Cl	H	H	Cl	87%
4q	H	Cl	H	Cl	H	62%
4r	H	Cl	Cl	H	H	35%
4s	H	Cl	H	H	F	55%
4t	H	Cl	H	H	Br	62%
4u	H	Cl	H	H	OCH_3	56%
4v	H	Cl	H	H	CH_3	59%
4w	H	Cl	H	CH_3	H	68%
4x	H	Cl	CH_3	H	H	32%

Scheme 2. Reagents and conditions: (a) Ac_2O, NaOH, H_2O; (b) BnBr, K_2CO_3, acetone, NaOH; (c) $(COCl)_2$, DMF, CH_2Cl_2; (d) DIEA, 3-fluoro benzenediamine, 3-chloro-1,2-benzenediamine, or 4-chloro-1,2-benzenediamine, CH_2Cl_2; (e) NaOH, H_2O, THF; (f) H_2, 10 wt% Pd/C, MeOH; (g) $LiAlH_4$, THF, reflux.

prior to conversion to the corresponding acid chloride. The acid chloride **6** was then treated with either 3-fluoro benzenediamine, 3-chloro-1,2-benzenediamine, or 4-chloro-1,2-benzenediamine to provide the corresponding amides **4a**-**4l**. The use of 3-fluoro benzenediamine (X = F) provides a direct analog to ester **1** with a 3-fluoro substituent.



Scheme 3. Reagents and conditions: (a) PBr_3, CH_2Cl_2, 0 °C – room temperature, 18 hr, 96%; (b) Et_3N, 3-mercaptophenol, CH_2Cl_2, 0 °C – room temperature, 18 hr, 100%; (c) mCPBA, 0 °C, 30 min, 54%; (d) mCPBA, 0 °C, 30 min, 71%.

The 4-fluoro and 4-chloro benzenediamines were used as those substitution patterns are more readily available. Deprotection of the acetate or benzyl ether followed by reduction with $LiAlH_4$ provided amines **4m**-**4x**.

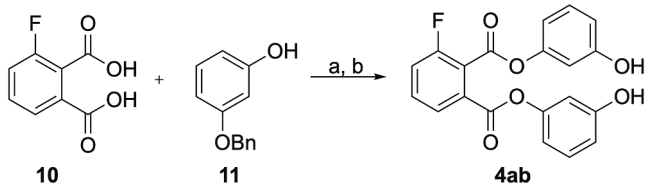
Sulfoxides and sulfones have previously been used as carbonyl isosteres in a series of HCVNS5B polymerase inhibitors.[22] To synthesize these isosteres the known dibromide **9**[23] was prepared from the diol **8** using PBr_3. The alkylation of 3-mercaptophenol with dibromide **9** in the presence of Et_3N provided thioether **4y**. By regulating the stoichiometry of *m*CPBA, **4y** was oxidized to the sulfoxide **4z** or the sulfone **4aa** (Scheme 3).

Another carbonyl isostere prepared was the inverted ester of **1**. As an ester, it will be readily hydrolyzed, it will however provide information regarding the optimal location of the carbonyl. Along with the amide and ester isosteres, inverted isosteres have been examined in the studies of meperidine analogs.[24] As shown in Scheme 4, 3-fluorophthalic acid (**10**) was coupled with the mono benzylated resorcinol **11** that was prepared using the procedure from Zhang.[25] The resulting ester was then deprotected via hydrogenolysis to obtain ester **4ab**.

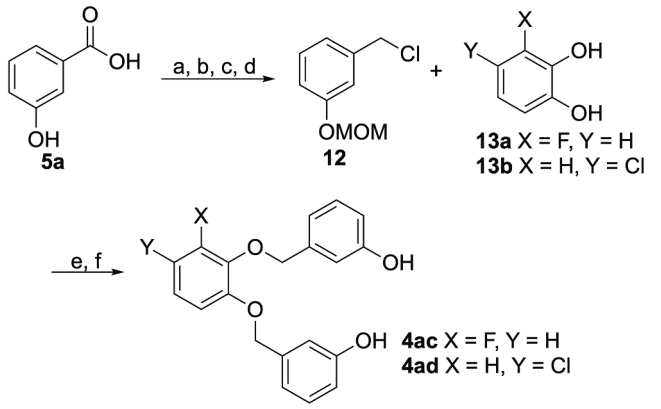
The next set of isosteres involved the removal of the carbonyl group. The resulting compounds will not undergo hydrolysis so they should be much more stable in serum than their ester counterparts. As shown in Scheme 5, 3-hydroxybenzoic acid (**5a**) was converted to the chloride **12** via a 4-step sequence of esterification, MOM-protection, reduction, and chloride formation. Alkylation of catechols **13a-b** with chloride **12** followed by deprotection provided ether derivatives **4ac** and **4ad**.

An inverted ether derivative was prepared as shown in Scheme 6. Phenol **14** was prepared from resorcinol and further alkylated with dibromide **9** followed by deprotection to provide target compound **4ae**.

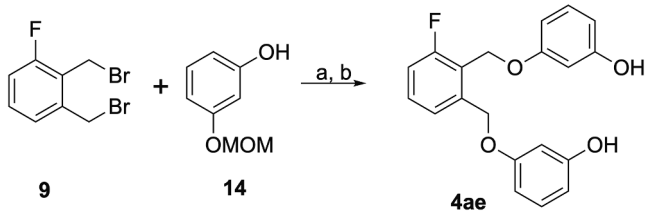
A final isostere was an all carbon linker between the central and pendent aromatic rings (Scheme 7). Wittig salt **15** was prepared by the addition of triphenylphosphine to dibromide **9** in methylene chloride. *n*-BuLi was added to **15** to produce the Wittig reagent followed by the addition of **16**. The product was a mixture of *cis* and *trans* isomers.



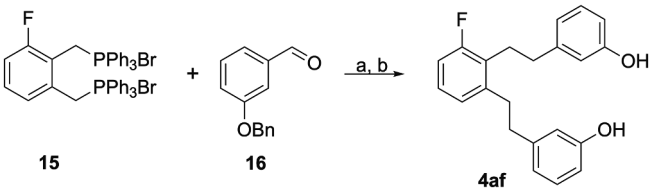
Scheme 4. Reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, 12 hr, 59%; (b) H_2, 10 wt% Pd/C, MeOH, 18 hr, 87%.



Scheme 5. Reagents and conditions: (a) H_2SO_4, MeOH, 5 hr, 91%; (b) MOMCl, K_2CO_3, DMF, 0 °C, 4 hr, 85%; (c) $LiAlH_4$, THF, 0 °C, 1 hr, 99%; (d) MsCl, Et_3N, CH_2Cl_2, 0 °C – room temperature, 18 hr, 84%; (e) K_2CO_3, DMF, 80 °C, 78–80%; (f) HCl, MeOH, 50 °C, 79–92%.



Scheme 6. Reagents and conditions: (a) K_2CO_3, DMF, 80 °C, 64%; (b) HCl, MeOH, 50 °C, 76%.



Scheme 7. Reagents and conditions: (a) *n*-BuLi, 0 °C – room temperature, 18 hr, 79%; (b) H_2, 10 wt% Pd/C, THF, 18 hr, 50%.

Reduction of the isomeric olefins along with the deprotection of the benzyl ethers was accomplished via catalytic hydrogenation to obtain compound **4af**.

The first set of compounds synthesized were simple isosteres of **1**. For these compounds, the fluorine and pendent aryl groups are arranged in positions-2,3,4 (Fig. 1) about the central aromatic ring. Table 1 shows the results of the assays performed. All compounds were evaluated in H1299 cells at a concentration of 30 μM.[27,28]

Amide **4a** and the corresponding amine **4m** are direct analogues of ester **1**. Amide **4a** showed a significant decrease in both glucose uptake inhibition and cell growth inhibition relative to ester **1**. The corresponding amine **4m** showed a similar loss of activity. As previously stated, modification of the halogen and its placement on the central ring was varied due to availability of the starting materials. The 4-fluoro and 4-chloro analogues of amide **4a** (**4b** and **4c**) showed improved glucose uptake inhibition but even more pronounced loss of cell growth inhibition. The corresponding amines **4n** (replacement of 3-F with 4-F) showed a moderate loss of activity in glucose uptake inhibition but a substantial increase in cell growth inhibition. It is significant that the glucose uptake and cell growth assays showed similar levels of inhibition indicative of glucose uptake inhibition causing the cell growth inhibition. Replacement of the 4-F with a 4-Cl (**4o**) again improved both the glucose uptake inhibition and cell growth inhibition.

Table 1
Activity of initial isosteres of **1***.

Compound #[26]	Glucose uptake inhibition (%)[27]	Cell growth inhibition (%)[28]
1 (WZB117)	92.8 ± 0.1	56.9 ± 9.0
4a	20.0 ± 1.0	33.4 ± 7.8
4b	89.7 ± 2.5	5.7 ± 2.1
4c	83.7 ± 0.7	5.9 ± 1.4
4m	54.8 ± 5.3	5.0 ± 16.9
4n	39.2 ± 0.8	37.7 ± 8.4
4o	52.2 ± 9.7	56.6 ± 11.2
4y	98.1 ± 0.2	−2.0 ± 20.2
4z	17.2 ± 4.5	0.2 ± 4.5
4aa	61.4 ± 9.4	11.8 ± 2.2
4ab	78.2 ± 1.3	15.3 ± 11.5
4ac	92.6 ± 2.2	10.6 ± 2.0
4ad	91.2 ± 0.8	14.3 ± 2.7
4ae	89.5 ± 2.9	−1.4 ± 17.4
4af	60.7 ± 5.4	−22.7 ± 8.6

*All compounds were tested at a concentration of 30 μM in H1299 cells.

Replacement of the ester functionality with a sulfide (**4y**) sulfoxide (**4z**) or sulfone (**4aa**) all led to compounds with a poor activity profile. While both the sulfide (**4y**) and sulfone (**4aa**) showed good to moderate glucose uptake inhibition, neither had any significant cell growth inhibition. The sulfoxide had little activity in either assay.

The inverted ester **4ab** showed moderate activity at inhibition of glucose uptake but rather poor inhibition of cell growth. Similarly, the ether **4ac**, it's 4-Cl variant **4ad**, and the inverted ether **4ae** showed good levels of glucose uptake inhibition but poor inhibition of cell growth. The last isostere, the all carbon chain **4af** again showed reasonable activity in glucose uptake inhibition but no activity inhibiting cell growth.

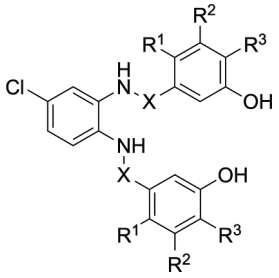
Relatively poor correlation was found between the glucose uptake assay and the cell viability/proliferation assay. Several factors contribute to the poor correlations. First, the glucose uptake assay is a 50 min acute assay while the cell viability/proliferation assay is a 24 hr chronic assay. It is well known that H1299 cells express different GLUTs at different levels and these GLUTs play somewhat different roles in the overall glucose metabolism.[29] These compounds are likely to inhibit GLUT-mediated glucose transport with varying potency, resulting in different cancer cell growth inhibitory activities.

Additionally, most cancer cell lines, including H1299 cells used in this study, exhibit macropinocytosis, through which glucose can be taken into cancer cells via bypassing GLUTs, weakening the correlation. Nevertheless, our ultimate goal of this study was to identify those compounds that reduce cancer cell viability/proliferation through inhibiting GLUTs, justifying the sequential use of the two assays in the study.

Of all of the isosteres prepared amide **4a** and amine **4n** and **4o** showed the closest correlation between cell growth inhibition and glucose uptake inhibition. While their overall activity was lower than ester **1**, we felt that additional modifications should yield improved activity.

Based on these initial results a series of additional amides and amines were prepared to optimize the activity of **4n**. Table 2 shows the activity of the amides and amines synthesized. For both the amide and amine derivatives analogs with a chloro-substitution at positions R^1, R^2, and R^3 were prepared in order to identify initial structure activity relationships relative to position around the phenolic ring. Amide derivatives **4d** (R^3 = Cl) showed a relatively similar inhibition of glucose uptake and cell growth inhibition relative to the unsubstituted **4b**. Amide **4e** (R^2 = Cl) showed a decrease in glucose uptake and an increase in cell growth inhibition. Significantly the levels of glucose uptake inhibition and cell growth inhibition were relatively similar. Amide **4f** showed a similar pattern as both **4b** and **4d**. The

Table 2
Activity of additional amide and amine derivatives*.



Compound #[26]	X	R^1	R^2	R^3	Glucose uptake inhibition (%)[27]	Cell growth inhibition (%)[28]
4b	CO	H	H	H	89.7 ± 2.5	5.7 ± 2.1
4o	CH_2	H	H	H	52.2 ± 9.7	56.6 ± 11.2
4d	CO	H	H	Cl	85.2 ± 2.4	19.0 ± 8.0
4e	CO	H	Cl	H	60.6 ± 1.7	61.3 ± 7.9
4f	CO	Cl	H	H	89.9 ± 2.0	45.3 ± 7.4
4g	CO	H	H	F	47.8 ± 6.5	25.7 ± 10.1
4h	CO	H	H	Br	97.3 ± 0.4	54.6 ± 4.2
4i	CO	H	H	OCH_3	39.5 ± 3.6	26.0 ± 14.0
4j	CO	H	H	CH_3	84.8 ± 1.2	55.0 ± 7.0
4k	CO	H	CH_3	H	61.4 ± 12.1	84.7 ± 0.8
4l	CO	CH_3	H	H	21.0 ± 8.2	21.0 ± 2.3
4p	CH_2	H	H	Cl	52.4 ± 10.9	70.9 ± 11.7
4q	CH_2	H	Cl	H	81.7 ± 4.5	47.6 ± 7.2
4r	CH_2	Cl	H	H	80.7 ± 1.2	70.8 ± 4.2
4s	CH_2	H	H	F	24.1 ± 1.6	22.1 ± 7.4
4t	CH_2	H	H	Br	69.1 ± 1.1	74.1 ± 7.0
4u	CH_2	H	H	OCH_3	42.2 ± 4.6	22.0 ± 9.5
4v	CH_2	H	H	CH_3	93.7 ± 4.5	77.5 ± 7.1
4w	CH_2	H	CH_3	H	75.0 ± 5.3	60.4 ± 8.2
4x	CH_2	CH_3	H	H	82.9 ± 1.1	46.3 ± 1.3

*All compounds were tested at a concentration of 30 μM in H1299 cells.

corresponding amines showed a rather different pattern with amine **4p** (R^3 = Cl) showing no change in glucose uptake inhibition and an increase in cell growth inhibition relative to amine **4o**. Amine **4q** showed an increase in glucose uptake inhibition and a small decrease in cell growth inhibition with respect to its amide homolog and the unsubstituted amine. Amine **4r** (R^1 = Cl) showed increases in both glucose uptake inhibition and cell growth inhibition. With these somewhat confusing directions as to which additional analogs to prepare the corresponding derivatives in which R^1, R^2, or R^3 = CH_3 were also prepared and evaluated.

The amide derivatives in which R^1, R^2, or R^3 = CH_3, **4j**, **4k**, and **4l**, showed a somewhat similar pattern as the corresponding chlorine-substituted amide, **4d**, **4e**, and **4f**. These compounds showed some improvement in either glucose uptake inhibition or cell growth inhibition. An exception being **4l** (R^1 = CH_3), this compound showed a decrease in both glucose uptake inhibition and cell growth inhibition albeit with both values being equivalent. The corresponding amine derivatives **4v**, **4w**, and **4x** showed a similar pattern. Amine **4v** proved to be an exception with improved and equivalent values for both glucose uptake inhibition and cell growth inhibition.

With an improved analog **4v** identified a small series of methyl replacements were again prepared and evaluated with the methyl replaced by fluorine, bromine, and methoxy. All of the amide analogues (**4g**, **4h**, **4i**) bearing these replacements provided mixed results in terms of glucose uptake inhibition and cell growth inhibition. Amine analog **4s** (R^3 = F) showed substantially reduced effectiveness, albeit roughly equivalent, in both glucose uptake inhibition and cell growth inhibition. Replacement of the methyl at R^3 with a bromine (**4t**) provided a compound with a similar activity profile as the parent compound. Replacing the methyl group with a methoxy (**4u**) however substantially reduced both glucose uptake inhibition and cell growth inhibition.

The simple isosteric replacement of the ester bond did not lead to any compounds with similar activity in both glucose uptake inhibition and cell growth inhibition. Modification of the phenolic ring of the amide/amine series did lead to an improved set of analogs. The best compound (**4v**) showed significantly better stability in human serum. To determine the compound stability, 30 μM of **1** and **4v** were incubated in serum-containing cell culture medium DMEM at 37 °C for different durations (0, 1, 2, 4, 6, 9, 12, 24, 48 and 72 h). After the incubation, 100 μL of compound-containing media were used to treat A549 cells at 37 °C. After incubation for 24 hrs, cell viability/proliferation was measured by a Resazurin assay.[30] The assay revealed that **1** started to lose its cancer cell inhibitory activity at 12 hrs while **4v** retained its full activity even after the 72 hr incubation, indicating that **4v** is at least 5 times more stable than **1**.

In addition, this improved amine analog (**4v**) showed improved activity in both glucose uptake inhibition and cell growth inhibition. Compound **4v** has IC_{50} values in H1299 cells for glucose uptake inhibition of 1.9 ± 0.3 μM and for cell growth inhibition an IC_{50} of 7.0 ± 0.6 μM. Future work on optimization of this class of compounds as well as determining their in vivo efficacy will be reported in due course.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127406.

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26. The identity of all compounds were verified by ^{1}H-NMR, ^{13}C-NMR and HRMS. All compounds were purified to > 95% as determined by HPLC.
27. The glucose uptake assay and cell growth inhibition assay were conducted as previously described.8 Before the glucose uptake assay, H1299 cancer cells were incubated with serum-free cell culture media for 2 hours and then KRP buffer for 30 minutes. The incubated H1299 cancer cells were treated with or without the compounds (30 μM) for 10 minutes followed by the addition of 37 MBq/L [^{3}H]2-deoxyglucose and 1 mmol/L regular glucose in KRP buffer for glucose uptake for 30 minutes. The cells were then washed twice with ice-cold PBS and lysed using 0.1 mM NaOH. The radioactivity was measured using Beckman Coulter Scintillation counter LS6500. Each experimental condition was performed in triplicates, quadruplets or hexads (or as mentioned otherwise), and the experiment was repeated at least once. Data is reported as mean ± standard deviation and analyzed using Student's t-test or one-way ANOVA, whichever is appropriate. $P \leq 0.05$ was considered significant. *, $P \leq 0.05$, **, $P \leq 0.01$, and ***, $P \leq 0.001$, and ****, $P \leq 0.0001$.".
28. The cell growth inhibitory assay was performed as follows. H1299 cancer cells were seeded at a density of 5,000 per well in 96-well plates and incubated with or without compounds (30 μM) in hexads for 48 hrs. DMSO (< 0.5%v/v) was used as solvent and control. After incubation, MTT was added and incubated with cells for 4 hours. Cell growth rates were measured using a Biotek Cytation3 microplate reader to measure the absorbance of MTT. The wavelength were measured was 570 nm. Each experimental condition was performed in triplicates, quadruplets or hexads (or as mentioned otherwise), and the experiment was repeated at least once. Data is reported as mean ± standard deviation and analyzed using Student's t-test or one-way ANOVA, whichever is appropriate. $P \leq 0.05$ was considered significant. *, $P \leq 0.05$, **, $P \leq 0.01$, and ***, $P \leq 0.001$, and ****, $P \leq 0.0001$.".
29. O'Byrne KJ, Baird AM, Kilmartin L, Leonard J, Sacevich C, Gray SG. *Cancers.* 2011;3:1550–1565.
30. Compounds at 30 μM were pre-incubated in A549 cells; DMEM cell culture medium containing 10% FBS and 1% Pen/Strp at 37°C for different duration (0,1, 2, 4, 6, 9, 12, 24, 48 and 72 hours). 5 x 10^{3} A549 cells were grown in DMEM cell culture media. After overnight incubation, cells were washed with PBS 2 times. Then, pre-incubated compound containing DMEM was added to cells (100 μl per well) for 24 hours. Resazurin was then added for 30 minutes. Absorbance values were measured at Ex 560 nM and Em 590 nM. Mock (DMSO) treated cells were used as controls to normalize all of the data points.