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Novel inhibitors of basal glucose transport as potential anticancer agents

Weihe Zhang [a], Yi Liu [b,c], Xiaozhuo Chen [b,c,d,e], Stephen C. Bergmeier [a,*]

[a] Department of Chemistry & Biochemistry, Ohio University, Athens, OH 45701, USA
[b] Department of Biological Sciences, Ohio University, Athens, OH 45701, USA
[c] Program in Molecular and Cellular Biology, Ohio University, Athens, OH 45701, USA
[d] Department of Biomedical Sciences, Ohio University, Athens, OH 45701, USA
[e] Edison Biotechnology Institute, Ohio University, Athens, OH 45701, USA

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ABSTRACT

Cancer cells commonly show increased levels of glucose uptake and dependence. A potential strategy for the treatment of cancer may be the inhibition of basal glucose transport. We report here the synthesis of a small library of polyphenolic esters that inhibit basal glucose transport in H1299 lung and other cancer cells. These basal glucose transport inhibitors also inhibit cancer cell growth in H1299 cells, and these two activities appear to be correlated.

Cancer has overtaken cardiovascular diseases as the #1 killer in the USA and an estimated 565,650 Americans died from it in 2008 alone.[1] Five year survival rates for some cancers have significantly improved in the past two decades while those of other cancers, such as pancreatic and lung cancer, remain disappointingly low.[2] A common feature of all cancers is their increased glucose uptake and dependence on glucose as a preferred source for energy and biosynthesis.[3] PET scan studies have correlated increased tumor aggressiveness and poor prognosis with upregulated levels of glucose uptake, glucose transporters, and glucose metabolism.[3–5] The glucose transporter 1 (Glut-1) is responsible for basal glucose transport in all cell types,[6] and its expression level is correlated with invasiveness and metastasis potentials of cancers.[7–9]

Cancer cells, because of their faster proliferation rates, are predominantly in a hypoxic (low oxygen) state. Therefore, cancer cells use glycolysis (lactate formation), rather than oxidative phosphorylation in mitochondria used by normal cells, as their predominant glucose metabolism pathway.[10] Such a glycolytic switch not only gives cancer higher potentials for metastasis and invasiveness,[11] but also increases cancer's vulnerability to external interference in glycolysis since cancer cells are 'addicted' to glucose and glycolysis.[3,10,12] It was found that cancer cells were induced to die when glucose was withdrawn,[13] indicating that cancer cells are very sensitive to glucose concentration changes and prone to apoptosis-induction when glucose is limited to cancer cells.

Ample evidence indicates that cancer cells are more sensitive to glucose deprivation than normal cells.[10,11,14] Numerous studies strongly suggest that inhibition of basal glucose transport induces apoptosis and blocks cancer cell growth. Inhibitors to various enzymes involved in glycolysis, have been used to inhibit differing steps in the glycolysis process, and shown to have significant anticancer efficacies. The glycolytic enzymes that have been targeted include: hexokinase;[15] ATP citrate lyase;[16] and pyruvate dehydrogenase kinase (PDK).[17,18] Among glycolysis inhibitors tested, 3-bromopyruvate and a hexokinase inhibitor were found to completely eradicate advanced glycolytic tumors in all treated mice.[19] Compounds targeting (inhibiting) mitochondrial glycolytic enzyme lactate dehydrogenase A (LDH-A) have shown significant anticancer activity both in vitro and in vivo.[20,21] 2-DG, the tracer used in PET scans for locating metastasis, has been used as a glucose competitor and a glycolytic inhibitor in anticancer clinical trials.[22,23] These and other related studies also showed that these inhibitors use apoptosis-induction as their anticancer mechanism.[16,17,20,21] The observation that glycolysis inhibitors cause more cancer cell growth inhibition than normal cell growth inhibition has been attributed cancer cells preference for glucose as an energy source and upregulation of glycolysis relative to normal cells.[11,12]

It has recently been reported that the addition of anti-Glut-1 antibodies to various lung and breast cancer cell lines significantly reduced the glucose uptake rate and proliferation of cancer cells, leading to induction of apoptosis.[24] Furthermore, the antibodies potentiated the anticancer effects of cancer drugs such as cisplatin, paclitaxel, and gefitinib.[24] These results clearly indicate that agents

* Corresponding author. Tel.: +1 740 517 8462; fax: +1 740 593 0148.
E-mail address: bergmeis@ohio.edu (S.C. Bergmeier).

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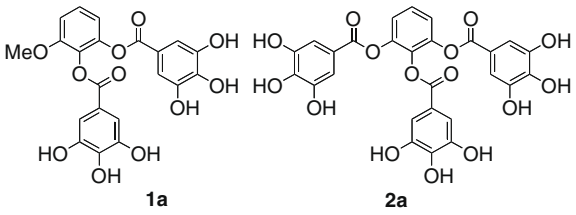


Figure 1. Initial glucose transport inhibitors **1a** and **2a**.

Table 1
Glucose transport inhibitory activity (%) of **1a** and **2a** in different cancer cell lines

Compound	Hela	RKO	MCF-7
1a	46.3 ± 3.3	57.2 ± 4.4	33.0 ± 0.5
2a	36.0 ± 4.9	58.1 ± 0.1	60.4 ± 2.4

that inhibit Glut-1-mediated glucose transport may work alone or in combination with other anticancer therapeutics to inhibit cancer cell growth and induce apoptosis in cancer cells. These findings are further supported by two recent publications in which glucose transport inhibitor fasentin was found to sensitize cancer cells to undergo apoptosis in the presence of the death ligand FAS[25] and anticancer compound apigenin was found to down-regulate Glut-1 at mRNA and protein levels.[26] Down regulation of Glut-1 was proposed as the potential anticancer mechanism for apigenin.[26] These reports indicate that glucose transport inhibitors are also likely to sensitize and synergize with other anticancer drugs to further enhance anticancer efficacy of the drugs.

We initially prepared compounds **1a** and **2a** as potential antidiabetic analogs of α-PGG[27–29] (Fig. 1). Given the tight SAR of this class of compounds, we hypothesized that a more rigid scaffold (i.e., a benzene ring) might enhance the activity. Rather than possessing insulin-like activity, these two compounds were surprisingly and serendipitously found to inhibit basal glucose transport on cervical (Hela), colon (RKO), and breast (MCF-7) cancer cells at a concentration of 30 μM (Table 1).

1a and **2a** also inhibited basal glucose transport in H1299 cells by 58.4 ± 6.3% and 86.1 ± 1.0%, respectively (Table 2), as measured by a standard glucose uptake assay[27,29] compared to non-compound treated cells controls (considered as 0% inhibition). Tested in an MTT cell proliferation assay in H1299 cells, their inhibitory activities on cancer cell growth were found to be 36.0 ± 6.1% and 39.9 ± 5.0%, respectively (non-compound treated cell controls were considered as 0% inhibition).

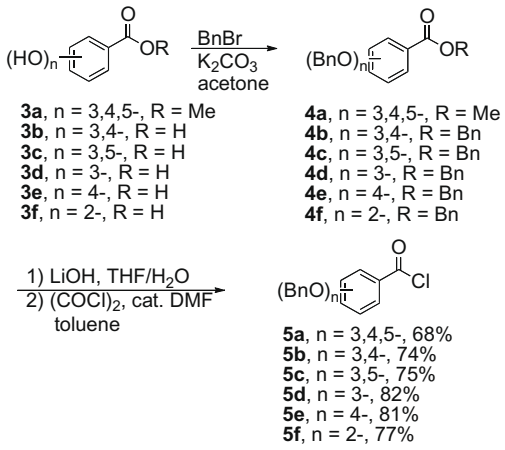
Given the potential utility of the inhibition of glucose transport for development of novel anticancer agents, we investigated structure–activity relationships of these compounds as both inhibitors of glucose transport and cancer cell proliferation. Based on these two compounds we have prepared a number of derivatives in order to understand the need for the trihydroxyphenyl ester and the need for three of these esters on the central aromatic ring.

The desired analogs were prepared by the acylation of a series of di- and tri-hydroxy benzenes with a group of substituted benzoyl halides. We have chosen a group of mono-, di-, and trihydroxybenzoyl halides as well as methoxybenzoyl halides as our acylating agents. The synthesis of the hydroxybenzoyl halides is outlined in Scheme 1. Commercially available phenols **3a–f** were perbenzylated and the resulting esters hydrolyzed and the acid

Table 2
Compounds prepared, their induced inhibitory activities in basal glucose transport and cell growth in H1299 lung cancer cells

Compound #	Ar/Ar′	X	Y	Yield[a] (%)	Glucose transport inhibition[b] (%)	Cell growth inhibition[b] (%)
1a	3,4,5-$(OH)_3$–C_6H_2	OMe	H	69	58.4 ± 1.0[c]	36.0 ± 6.1
2a	3,4,5-$(OH)_3$–C_6H_2	—	—	78	86.1 ± 1.0	39.9 ± 5.0
1b	3,4,5-$(OH)_3$–C_6H_2	H	Cl	69	84.4 ± 0.1	—
1c	3,4,5-$(OH)_3$–C_6H_2	F	H	78	81.1 ± 1.1	—
1d	3,4,5-$(OH)_3$–C_6H_2	H	H	65	32.1 ± 6.2	—
9a	3,4,5-$(OMe)_3$–C_6H_2	—	—	86	41.1 ± 1.5	—
7a	3,4,5-$(OMe)_3$–C_6H_2	OMe	H	90	33.3 ± 3.6	22.2 ± 3.4
7b	3,4,5-$(OMe)_3$–C_6H_2	H	Cl	89	1.7 ± 1.1	20.6 ± 2.1
7c	3,4,5-$(OMe)_3$–C_6H_2	F	H	87	7.9 ± 2.6	19.6 ± 3.6
9b	2,6-$(OMe)_2$–C_6H_3	—	—	69	75.8 ± 4.2	—
9c	3,4-$(OMe)_2$–C_6H_3	—	—	78	0 ± 3.9	10.0 ± 1.1
9d	3-(OMe)–C_6H_4	—	—	92	66.2 ± 1.8	—
7d	3-(OMe)–C_6H_4	OMe	H	96	32.1 ± 0.2	18.3 ± 4.3
2b	3,5-$(OH)_2$–C_6H_3	—	—	81	98.7 ± 0.8	41.0 ± 5.5
1e	3,5-$(OH)_2$–C_6H_3	OMe	H	78	69.4 ± 3.0	—
1f	3,5-$(OH)_2$–C_6H_3	H	Cl	87	95.2 ± 0.2	38.8 ± 6.9
1g	3,5-$(OH)_2$–C_6H_3	F	H	81	94.0 ± 0.8	35.0 ± 7.6
2c	3,4-$(OH)_2$–C_6H_3	—	—	81	94.7 ± 0.4	42.2 ± 6.1
1h	3,4-$(OH)_2$–C_6H_3	OMe	H	78	0	32.4 ± 2.7
1i	3,4-$(OH)_2$–C_6H_3	H	Cl	87	94.4 ± 0.4	45.2 ± 7.6
1j	3,4-$(OH)_2$–C_6H_3	F	H	81	88.7 ± 1.8	41.5 ± 5.5
2d	2-(OH)–C_6H_4	—	—	88	74.7 ± 2.0	—
1k	2-(OH)–C_6H_4	OMe	H	91	50.5 ± 7.6	18.0 ± 4.4
1l	2-(OH)–C_6H_4	H	Cl	92	51.6 ± 5.9	26.6 ± 2.3
1m	2-(OH)–C_6H_4	F	H	89	51.0 ± 6.6	—
2e	4-(OH)–C_6H_4	—	—	81	88.7 ± 2.5	34.8 ± 7.7
1n	4-(OH)–C_6H_4	OMe	H	78	86.5 ± 2.8	36.6 ± 6.7
1o	4-(OH)–C_6H_4	H	Cl	87	79.0 ± 8.7	—
1p	4-(OH)–C_6H_4	F	H	84	92.6 ± 0.7	35.9 ± 4.7
1q	4-(OH)–C_6H_4	H	H	82	57.5 ± 2.9	—
2f	3-(OH)–C_6H_4	—	—	81	99.7 ± 0.1	59.3 ± 4.9
1r	3-(OH)–C_6H_4	OMe	H	78	79.0 ± 8.0	—
1s	3-(OH)–C_6H_4	H	Cl	87	93.1 ± 1.7	44.5 ± 5.2
1t	3-(OH)–C_6H_4	F	H	81	92.8 ± 0.1	40.8 ± 5.6

[a] Overall yield from **6** or **8**.
[b] Untreated cells served as negative controls (0% inhibition).
[c] Data were presented as mean ± standard deviation.

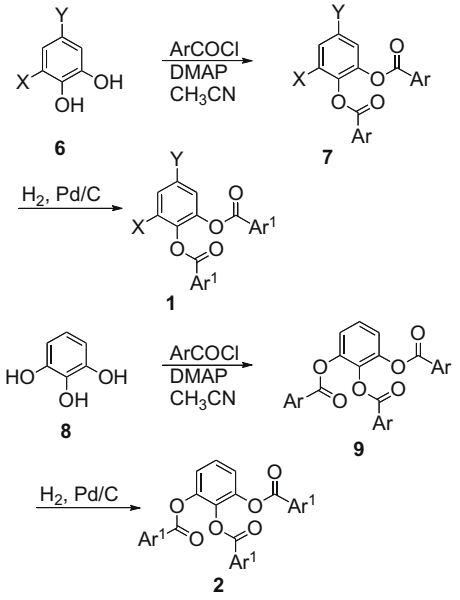


Scheme 1. Synthesis of the benzyloxybenzoyl chlorides **5**.

converted to the acid chlorides **5a**[29] and benzyloxybenzoyl chlorides **5b**,[30] **5c**, **5d**,[31] **5e**,[32] and **5f**.[33] The requisite methoxy-substituted benzoyl halides were prepared from the commercially available carboxylic acids.

In terms of the core aromatic ring, we have chosen pyrogallol **8** (present in **2a**) and 3-methoxycatechol **6a** (X = OMe, Y = H, present in **1a**). We have also chosen two halogen substituted phenols, **6b** (X = H, Y = Cl) and **6c** (X = F, Y = H) to provide a π-donor (similar to the methoxy group) but electron withdrawing group. We have included an unsubstituted catechol **6d** (X, Y = H). As shown in Scheme 2, each of these phenols was then coupled to each of the acid chlorides **5a**–**f** and several methoxy-substituted benzoyl chlorides. After coupling, the benzyloxy esters were deprotected via catalytic hydrogenation. The overall yields are shown in Table 2.

As shown in Table 2, compounds were tested in a standard glucose uptake assay. Briefly, H1299 cancer cells were treated with or without the compounds (30 μM) in triplicates for 10 min before the glucose uptake assay. Cellular glucose uptake was measured by incubating cells in the glucose-free KRP buffer with 0.2 Ci/mL [^{3}H]2-deoxyglucose (specific activity, 40 Ci/mmol) for 30 min in the absence or presence of compounds. After the cells were washed



Scheme 2. Synthesis of analogs **1**, **2**, **7**, and **9**.

with ice-cold PBS and lysed by 0.2 N NaOH, the cell lysates were transferred to scintillation counting vials and the radioactivity in the cell lysates was quantified by liquid scintillation counting. Cell growth measurements were performed using an MTT assay in hexads in a 96-well tissue culture plate with 5,000 cells plated in each well. The cells were incubated in presence or absence of the compounds (30 μM) for 48 h. After incubation, cell growth rates were assayed using a 96-well Spectramax absorbance/fluorescence plate reader (Molecular Devices).

In comparing the original compounds **1a** and **2a** to derivatives in which the core aromatic ring was substituted with a fluorine or chlorine (**1b** and **1c**), both of these compounds have similar activity in the glucose transport inhibition assay as compound **2a** and were significantly better than **1a**. Clearly the halogen substitution on the core aromatic ring is important. An unsubstituted core aromatic ring **1d** showed lower levels of inhibition relative to **1a** and **2a**. In order to determine the necessity of the phenolic hydroxyl groups we prepared several derivatives of **1a** and **2a** in which the OH group was replaced with a methoxyl group (**9a**–**d**, **7a**–**d**). These compounds showed uniformly lower levels of inhibition of glucose transport. Only compounds **9b** and **9d** (2,6-dimethoxybenzoyl and 3-methoxybenzoyl) showed moderate levels of inhibition.

We next systematically removed phenolic hydroxyl groups preparing a series of di-hydroxyl and mono-hydroxyl derivatives. The 3,5-dihydroxy and 3,4-dihydroxyl derivatives overall showed good levels of glucose transport inhibition. In both series the tribenzoyl derivatives (**2b** and **2c**) as well as the fluoro- and chloro-substituted derivatives showed >90% inhibition while the methoxy-substituted derivatives (**1e** and **1h**) showed little to no inhibition. All of the compounds showing >90% glucose transport inhibition also show ~40% decrease in cancer cell growth rate.

Removing an additional hydroxyl group provided a set of mono-hydroxyl compounds at the 2-, 3-, and 4-positions. The 2-hydroxyl series showed uniformly poorer inhibition of glucose transport. The 4-hydroxyl series while showing poorer inhibition of glucose transport did provide compounds with >85% inhibition of glucose transport. These compounds showed a >35% decrease in cancer cell

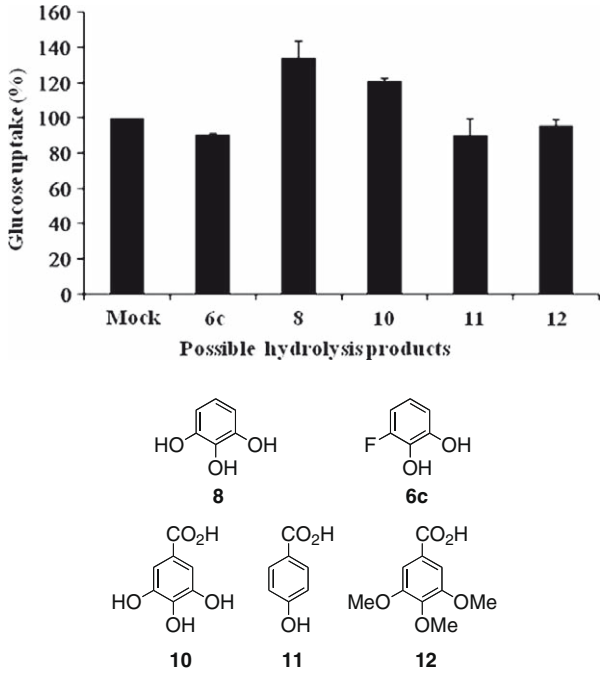


Figure 2. Glucose uptake results for the possible hydrolyzed products from **1p**, **9a**, and **2a**.

growth rate. The 3-hydroxyl series showed excellent inhibition of glucose transport with the tribenzoyl derivative **2f** showing >99% inhibition of glucose transport. This compound also showed the highest level of cell growth inhibition at ~60%. By analyzing all data in Table 2, it was found that the linear correlation coefficient $R = 0.817$ ($R^2 = 0.667$), indicating that approximately 2/3 (66.7%) of the inhibitory activity of cancer cell growth came from the inhibitory activity of basal glucose transport.

In general, the presence of a hydroxy group at the 3-position of the pendant benzoyl group is important for both inhibitions of glucose transport and cancer cell growth. This 3-hydroxy group can be attached to a chloro- or fluoro-substituted core benzene ring or be part of a tribenzoyl system. Unsubstituted or electron-donating substituents lead to significant decreases in the inhibition of glucose transport activity.

Given that our compounds are phenolic esters and hydrolysis may be facile we examined a few select hydrolysis products (**8**, **6c**, **10**, **11**, and **12**) for glucose uptake inhibition activity. As shown in Figure 2, none of these compounds had any glucose uptake inhibition activity. Similarly, none of these compounds showed any activity in anticancer screens (data not shown).

In summary, we have successfully synthesized a library of mutiphenolic ester compounds as novel inhibitors of basal glucose transport and anticancer agents with a potential new target, basal glucose transport. Further studies to confirm the mechanism of action and optimize these compounds are ongoing.

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