Oxime-based inhibitors of glucose transporter 1 displaying antiproliferative effects in cancer cells

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ABSTRACT

An analysis of the main pharmacophoric features present in the still limited number of inhibitors of glucose transporter GLUT1 led to the identification of new oxime-based inhibitors, which proved to be able to efficiently hinder glucose uptake and cell growth in H1299 lung cancer cells. The most important interactions of a representative inhibitor were indicated by a novel computational model of GLUT1, which was purposely developed to explain these results and to provide useful indications for the design and the development of new and more efficient GLUT1 inhibitors.

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Alterations of cancer cell glucose metabolism (Warburg effect) are now considered an emerging hallmark of cancer.[1] Hence, inhibition of the glycolytic processes associated with cancer cell viability currently represents a very promising strategy for the development of new cancer therapies.[2] The lower efficiency of glycolysis when compared to oxidative phosphorylation (OXPHOS) is counterbalanced by the overexpression of glucose transporters (GLUTs) and enzymes of glycolysis. This generally results in an enhanced glucose uptake by cancer tissues, which is exploited for diagnostic/imaging purposes with the use of the radiolabeled glucose analogue [^{18}F]fluorodeoxyglucose (FDG).[3] In humans, up to 14 GLUT family members have been identified so far, although GLUT1 and GLUT3 were principally found to be overexpressed in some carcinoma cell lines and, therefore, are directly linked to the Warburg effect.[4,5] In 2007, it was demonstrated that the use of antibodies against GLUT1 induced growth arrest and apoptosis in breast carcinoma, osteosarcoma, and non-small cell lung carcinoma (NSCLC) cancer cells.[6] Therefore, GLUT1 is currently being considered as an actual potential target to effectively tackle cancer. In spite of the growing interest toward this target, a quite limited number of GLUT1-inhibitors have been reported so far in literature,[2] including STF-31,[7] phloretin[8] and a series of hydroxylated phenyl esters,[9–11] such as WZB117 (Fig. 1).

We were particularly intrigued by the common pharmacophoric features encountered in the latter two classes of GLUT1-inhibitors and some oxime derivatives we had previously designed and synthesized as estrogen receptor (ER) ligands (Fig. 1),[12–14] mainly consisting in the presence of similarly-spaced peripheral "phenol-type" OH groups.

Curiously, other nuclear receptor binders, such as some thiazolidinediones showing PPARγ-agonist properties, were also reported to inhibit GLUT1.[15–17] Therefore, we decided to screen a focused collection of our oxime-based compounds (Fig. 1) for inhibition of glucose transport through GLUT1 and, consequently, for antiproliferative activity against H1299 cancer cells (NSCLC). These compounds were tested in a standard glucose uptake assay.[9–11] Briefly, H1299 cancer cells were treated with the compounds (30 μM) for 15 min before the glucose uptake assay. Cellular glucose uptake was measured by incubating cells in the glucose-free KRP buffer with 2-deoxy-D-[^{3}H]-glucose for 30 min in the absence or presence of compounds. After the cells were washed with ice-cold PBS and lysed by 0.2 M NaOH, the cell lysates were transferred to scintillation counting vials and the radioactivity in the cell lysates was quantified by liquid scintillation counting. The screening of this collection produced a list of oxime derivatives (**1–13**, Fig. 2), that proved to appreciably reduce glucose uptake at a 30 μM concentration, and some of them (**3**, **6**, **11–13**) displayed activities which were comparable to, or higher than, reference GLUT1 inhibitors, such as phloretin and WZB117 (Fig. 3, upper panel).

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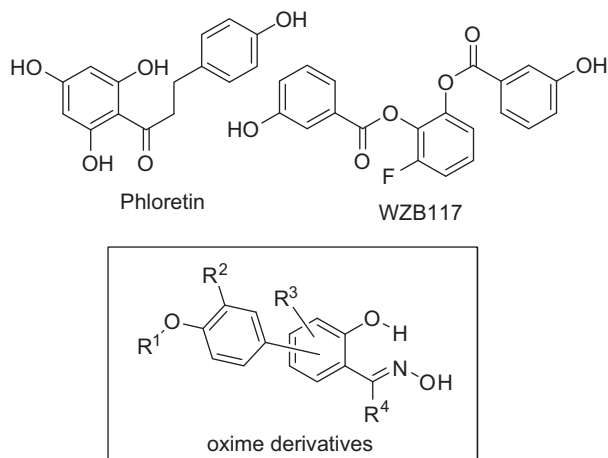 E-mail address: filippo.minutolo@farm.unipi.it (F. Minutolo).

Figure 1. Structural comparison of representative GLUT1-inhibitors and oxime derivatives.
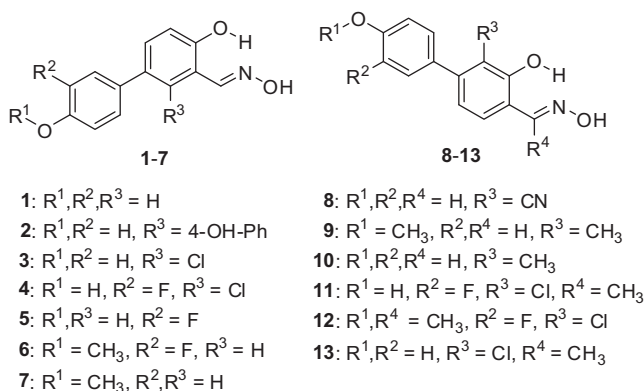

1: R¹,R²,R³ = H
2: R¹,R² = H, R³ = 4-OH-Ph
3: R¹,R² = H, R³ = Cl
4: R¹ = H, R² = F, R³ = Cl
5: R¹,R³ = H, R² = F
6: R¹ = CH₃, R² = F, R³ = H
7: R¹ = CH₃, R²,R³ = H

8: R¹,R²,R⁴ = H, R³ = CN
9: R¹ = CH₃, R²,R⁴ = H, R³ = CH₃
10: R¹,R²,R⁴ = H, R³ = CH₃
11: R¹ = H, R² = F, R³ = Cl, R⁴ = CH₃
12: R¹,R⁴ = CH₃, R² = F, R³ = Cl
13: R¹,R² = H, R³ = Cl, R⁴ = CH₃

Figure 2. Structures of oxime derivatives **1–13**, which were screened for inhibition GLUT1 and for antiproliferative effect in cancer cells.

These compounds (**1–13**) were then subjected to an antiproliferative assay. In this case cell growth measurements were performed using an MTT assay.[9–11] The cells were incubated in presence of the compounds (30 μM) for 48 h. The percentage values of viable cells associated to each compounds are reported in Figure 3 (lower panel). In this case a fraction of these compounds produced an appreciable inhibition of cell viability (**5–7**, **12**, **13**), which was comparable or higher than that of WZB117 (Fig. 3). On the basis of the combined results obtained in this preliminary screening, it became clear that aldoximes bearing the aryl substituent in the *meta*-position, such as compounds **5–7**, or ketoximes with the aryl substituent in the *para*-position (**12** and **13**), displayed the most interesting combined properties in terms of inhibition of glucose transport and of cell viability. Interestingly, the binding affinity of compounds **6**, **7**, **12**, and **13** for the estrogen receptors (the target they had initially designed to hit)[13] was previously found to be negligible, when compared to that reported for phloretin itself (hER-IC$_{50}$ = 300 nM),[18] thus conferring to these derivatives a well-defined preferential activity on GLUT1. On the contrary, oxime **5** had previously displayed a certain binding affinity for ERβ (K_d = 51 nM; RBA = 0.97%)[13] and, therefore, this compound did not progress in our screening funnel.

Hence, the effects of compounds **6**, **7**, **12**, and **13** on glucose uptake and cell viability were further studied at various concentrations, in order to measure their IC$_{50}$ values in both assays (Table 1). The values so obtained showed that these four oxime derivatives possess higher overall inhibitory properties against
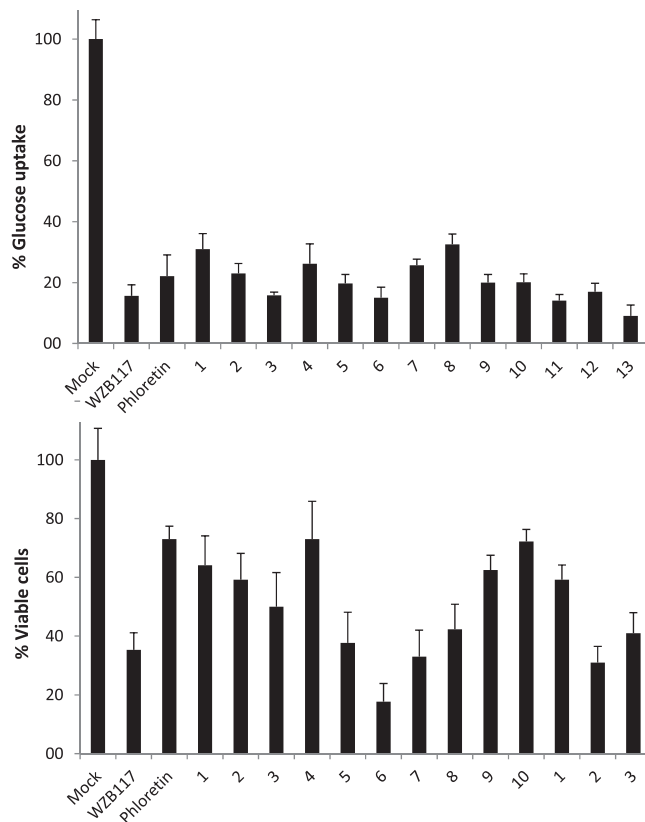

Figure 3. Inhibition % of glucose uptake (upper panel) and of cell viability after 48 h (lower panel) in H1299 lung cancer cells by compounds **1–13** and by reference inhibitor WZB117 and Phloretin at a 30 μM concentration.

glucose transport and cell proliferation than those displayed by reference GLUT1-inhibitor phloretin. Furthermore, the IC$_{50}$ values of all these compounds favorably compare to those obtained with GLUT1-inhibitor WZB117, thus confirming that the structural class of oxime derivatives may provide new and more potent GLUT1-inhibitors. The antiproliferative effect displayed by these compounds in H1299 lung cancer cells would support further investigation in vivo, in order to assess their selective toxicity in cancer cells. On the contrary, we dispute that toxicity in healthy cells in culture provides significant indications as regards tolerability in whole organisms. Consequently, we did not consider as relevant any in vitro assay in non-tumorigenic cell lines.

We then wanted to understand the way these compounds interact with GLUT1. Unfortunately there are no X-ray structures available of human GLUT1. However, the recently deposited structure of XylE,[19] an *Escherichia coli* homologue of GLUT1-4, showed the best

Table 1
Glucose uptake and cell growth inhibitory activities (IC$_{50}$) of compounds **6**, **7**, **12**, **13**, WZB117 and Phloretin in H1299 lung cancer cells

Compound	IC$_{50}$ value (μM)[a]	
	Glucose uptake	Cell viability
Phloretin	21.4 ± 5.1	54.0 ± 14.6
WZB117	10.9 ± 3.6	20.4 ± 4.8
6	8.5 ± 2.0	14.1 ± 4.8
7	23.4 ± 5.1	20.4 ± 5.4
12	15.5 ± 3.8	39.6 ± 11.8
13	10.6 ± 2.8	34.8 ± 5.8

[a] Values are reported as the mean ± the SD of 3 or more independent experiments. Data were analyzed and IC$_{50}$ determined by GraphPad Prism version 6.02 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com