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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Screening for anticancer properties of thiazolidinedione compounds in a galactose media metastatic breast cancer cell model

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Abstract

The metastatic tumors of breast cancer lead to the mortality of patients in part due to the failure of chemotherapeutics to reach the brain and emergence of drug resistance. With the increase in drug resistance, we focused on targeting mitochondrial metabolism, which was recently found to be a viable and novel drug target in breast cancer. Here we screened several ligands for anticancer activity in a phenotypic screen using the MTT dye as a marker for cell proliferation. MCF-7 breast cancer cells were treated with compounds in the presence of galactose to ensure mitochondrial interaction by the compounds. The lead compound of our group, NL-1, a thiazolidinedione (TZD), was found to inhibit cell proliferation with an IC_{50} of 7.1 μM, whereas its unsaturated derivative CI-987 was more potent with a IC_{50} of 3.3 μM. The TZDs were affected by substitution on both the aromatic ring and the TZD warhead. Taken together, these compounds can serve as lead structures for mitochondrial targeted drug discovery programs in breast cancer.

Keywords Warburg · Mitochondria · Glycolysis · Cisd1 · Cisd2 · Phenotypic screen

Introduction

Breast cancer has rapidly grown to be one of the greatest causes of cancer mortalities in women worldwide. According to Centers for Disease Control and Prevention, breast cancer is the most common cancer afflicting women in the United States. It is also the second leading cause of cancer fatalities among women. Targeting the mitochondrial function in cancer cells have gained support recently due to deeper insight into the metabolic difference between normal and cancerous cells (Martinez-Outschoorn et al. 2017). Cancer cells have been now shown to have a heterogeneity in the energy production, with either a propensity for using the glycolysis (Warburg) pathway or the oxidative phosphorylation (OXPHOS) pathway (Martinez-Outschoorn et al. 2017; Liberti and Locasale 2016). In this process, the cancer cells have an increased uptake of glucose, and the higher amounts of glucose are subsequently metabolized to yield higher amounts of lactic acid (Liberti and Locasale 2016). This higher consumption and fermentation of glucose happens in presence oxygen, and was termed "aerobic glycolysis." Based on these data, it has been suggested that targeting the mitochondria in cancer cells can be a viable strategy for synergistic treatment with the standard of care compounds (Liberti and Locasale 2016; Zhang and Yang 2013). In addition, studies have indicated that cancer resistance may in part be supported via mitochondrial changes in the cellular environment, suggesting that targeting mitochondria in cancer may be an attractive drug target (Peiris-Pages et al. 2019).

Our group has been developing compounds that can be used to alter metabolism in cells by targeting the mitochondrial function (Geldenhuys et al. 2010; Geldenhuys et al. 2016). We have focused on the thiazolidinedione (TZD) warhead, which is a critical moiety in the antidiabetic drug pioglitazone. Pioglitazone is a PPAR-γ agonist, but has been shown to also have anticancer activity (Urakami-Takebayashi et al. 2018; Kole et al. 2016; Frohlich and Wahl 2015). The efficacy of the TZD derivatives was tested on MCF-7 breast cancer cell model. To prevent the cancer cells from over-utilizing glucose and changing mitochondrial dynamics, the cells were cultured in a galactose medium. Utilizing galactose-rich media instead of glucose, forces the cells to their energy metabolism from glycolysis to the mitochondria for OXPHOS. This technique has been

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Table 1 Antiproliferation activity of the compounds tested

	Compound	IC_{50} (μM) MCF-7
1	NL-1	7.18
2	Epalrestat	407.90
3	A6355	81.12
4	SCF-2	26.93
5	NL-2	19.75
7	CI-987	3.68
8	NEO1-1	1.129
9	NEO1-2	1.127
10	5725195	4.84
11	5721666	4.41
12	5108116	1.18
13	5108007	12.50
14	5481970	ND
15	6009237	29.29
16	5739278	431.9
17	5109185	0.988
18	5182646	50.04
19	5236896	13.40
20	5108134	9.11
21	5236894	17.33
Pioglitazone		22.09
Doxorubicin		0.388

ND not determined

used in the past for identify the effect of compounds on mitochondrial activity. (Tsiper et al. 2012; Kamalian et al. 2015; Grimm et al. 2017; Sanuki et al. 2017) Few studies have been initiated to delineate the effects of TZDs on mitochondrial-based anticancer activity, which is the topic of this research report.

Materials and methods

All the TZD compound were purchased from Sigma Aldrich or Hit2lead and were ≥98% pure. The MTT dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was obtained from Sigma Aldrich. MCF-7 (ATCC HTB-22) cells were grown in Eagle's Minimum Essential medium (ATCC) supplemented with fetal bovine serum 10% v/v and penicillin/streptomycin 5% v/v, at 5% CO_2 and 37 °C. During the treatment phase, the media were replaced with DMEM (Gibco) without glucose and supplemented with 10 mM galactose.

MTT assay

Cells were seeded at 5000 cells per well in a clear 96-well plate, and the media changed to the 10 mM galactose DMEM. The next day (24 h), cells were treated with each compound in a dose-dependent manner, and allowed to grow for 72 h. Each compound was prepared as 10 mM stocks in DMSO, and the final concentration of DMSO in each well was 1%. On the final day (72 h), the media were removed using a vacuum aspirator, and then phosphate buffered saline (PBS) pH 7.4 with 1 mg/mL yellow MTT dye was added to each well, and incubated for 2 h. The PBS was then removed and the insoluble blue formazan crystals dissolved with 100 μL DMSO, by shaking the plate at 100 rpm, 37 °C for 30 min. Absorbance of the plate was measured at 570 nm using a BioTek Synergy 4 plate reader. Data were graphed in GraphPad Prism and the IC_{50} values determined using a one-site binding model.

Molecular modeling

Compounds were drawn in ChemDraw Prime (PerkinElmer) and saved as SDF files. These files were combined into a database with MOE 2019 (www.chemicalcompoutingroup.com) and molecular descriptors calculated based on both the 2D and 3D structures. Correlation graphs between molecular descriptors shown in Table 1 was made in OSIRIS DataWarrior QSAR package. Tautomers for NL-1 and CI-987 were analyzed with the program Tautomers (OpenEye Cheminformatics Software www.eyesopen.com).

Tautomerism study of CI-987

CI-987 was dissolved in DMSO as a 10 mM stock solution. In a UV transparent (Costar) 96-well plate, 100 μL of a 50 mM citrate buffer (pH 2), a phosphate buffer (pH 6 and 7.4), Tris buffer (pH 8), and borate buffer (pH 9) were incubated with either 1 μL CI-987 or DMSO. The DMSO curve was used as blank and subtracted from the CI-987 curve. UV-color spectrums were taken using a Synergy 4 plate reader (BioTek) scanning from 250 to 700 nm, and the data were graphed in GraphPad Prism. The maximal absorption of CI-987 (lamda max) was found to be 350 nm at pH 9, which was visible as a yellow color change.

Results and discussion

In this study, we evaluate a series of TZD compounds for anticancer activity in MCF-7 breast cancer cells specifically targeting the mitochondrial effects by using a galactose media-based protocol. A group of TZDs (which included rhodanine scaffolds) were tested as well as a control group five-membered ring system compounds. To test the effect of mitochondrial function, we replaced the high glucose concentration seen in most cell culture media with galactose,

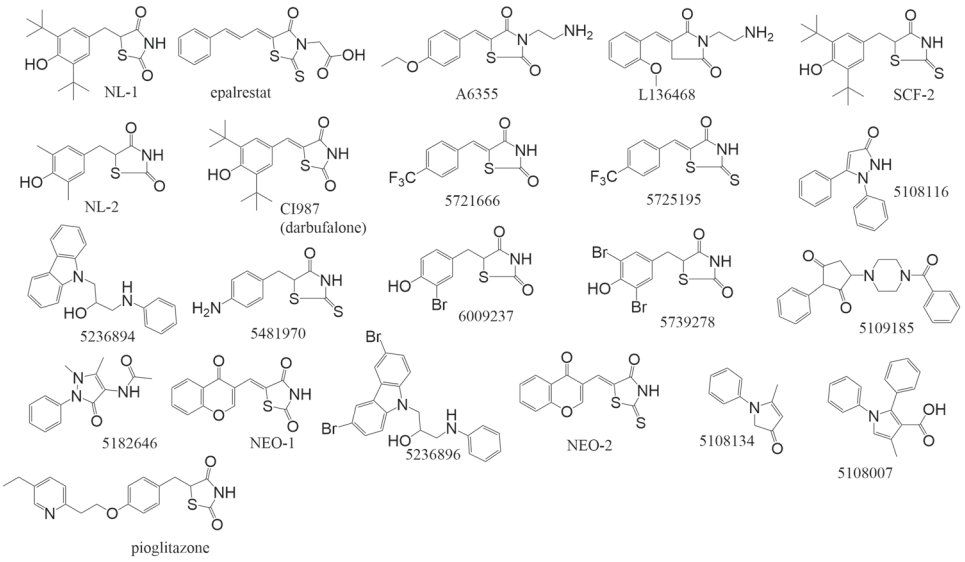

Fig. 1 Structures of the compounds discussed in the text

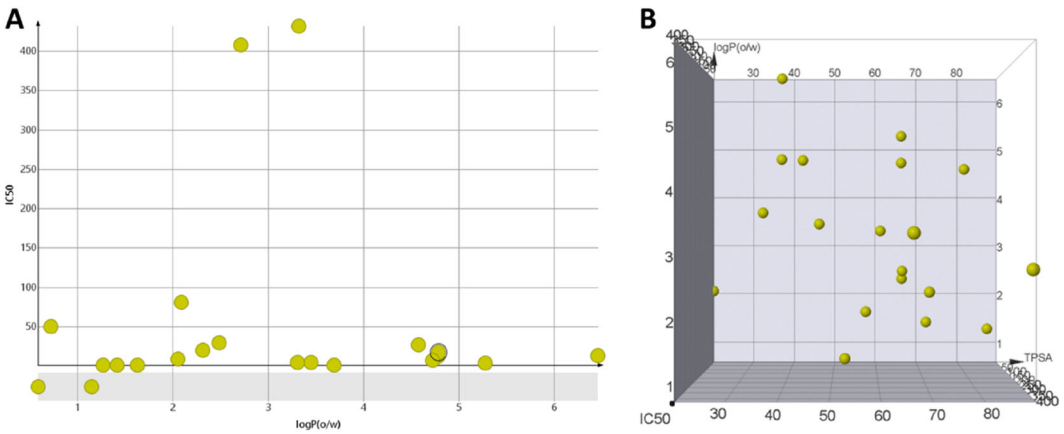

Fig. 2 The compounds evaluated in the phenotypic screen had a diverse logP and TPSA. **a** LogP diversity; **b** LogP, TPSA, and IC50

which forces the energy production of the cell via the OXPHOS pathways (Tsiper et al. 2012; Kamalian et al. 2015; Grimm et al. 2017; Sanuki et al. 2017; Aguer et al. 2011). This protocol has also been used in other studies to access the activity profile of new compounds on mitochondrial OXPHOS health (Tsiper et al. 2012; Kamalian et al. 2015; Grimm et al. 2017; Sanuki et al. 2017). The chemical structures of the compounds are shown in Fig. 1 and the result from the MTT assay is shown in Table 1. The chemical properties space of the compounds tested are shown in Fig. 2. For control compounds, we used the antidiabetic drug pioglitazone (Actos) and doxorubicin. The results of the effects the control compounds are shown in Fig. 3. As can be seen, pioglitazone had little effect on the number of cells after 72 h of incubation, while as expected doxorubicin significantly inhibited the proliferation over the 72-h period. For doxorubicin, the IC50 changed from 388 nM in high glucose to 602 nM in galactose media, while pioglitazone did not shown any significant effect on cell proliferation in the presence of high glucose, but in the presence of galactose was 22 µM.

The most potent TZD found in the current set was compound **9** ($IC_{50} = 1.127$ µM), while the most potent overall compound tested was the monoketone **17** ($IC_{50} =$ 0.988 µM). The lead compound of this study, NL-1, was designed by us in a previous to target the mitochondrial protein mitoNEET. The mitoNEET specific ligand, NL-1, has been previously developed that is devoid of the PPARγ activity of thiazolidinediones (Geldenhuys et al. 2010). NL-1 was found to improve survival of cardiac stem cells following oxidative stress in vitro as well as in an in vivo model of Zucker obese rats (Logan et al. 2015). In this current assay, NL-1 inhibited cell proliferation with an IC_{50} of 7.18 µM in the presence of galactose, while no effect was seen with high glucose media. Interestingly, the structural analog CI-987, which has an unsaturated bond, was more

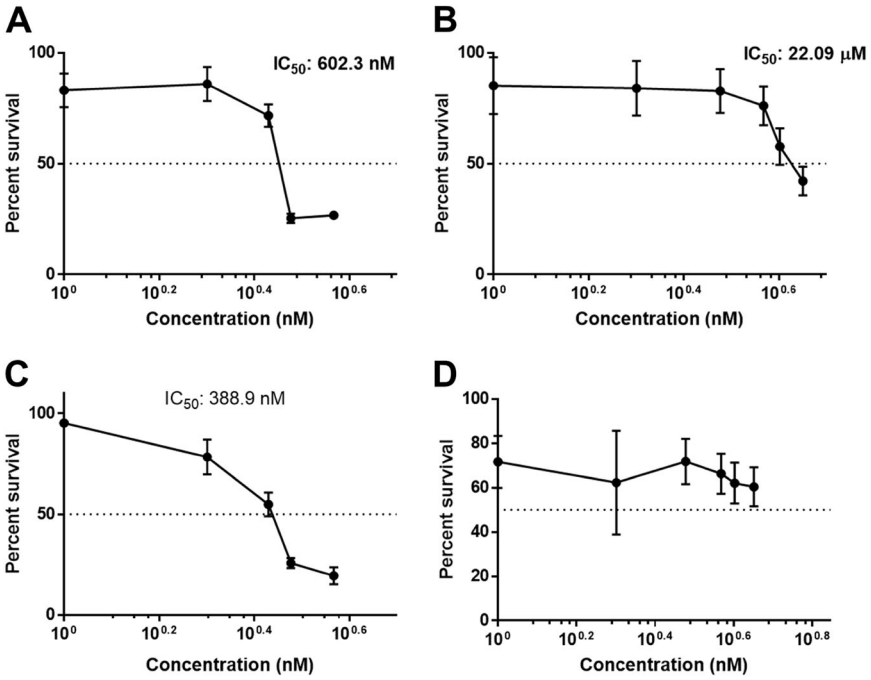

Fig. 3 Effect of galactose and glucose on cell proliferation in the presence of compounds. **a** doxorubicin in galactose media; **b** pioglitazone in galactose media; **c** doxorubicin in high glucose media; and **d** pioglitazone in high glucose media. Each data point represent average ± S.D. where $N = 8$

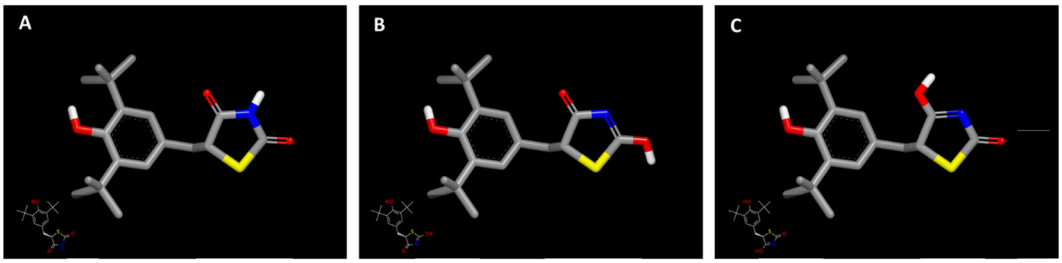

Fig. 4 Tautomers of NL-1. The tautomers of the TZD warhead reveal that a keto-enol state is possible and could contribute to the activity profile of NL-1

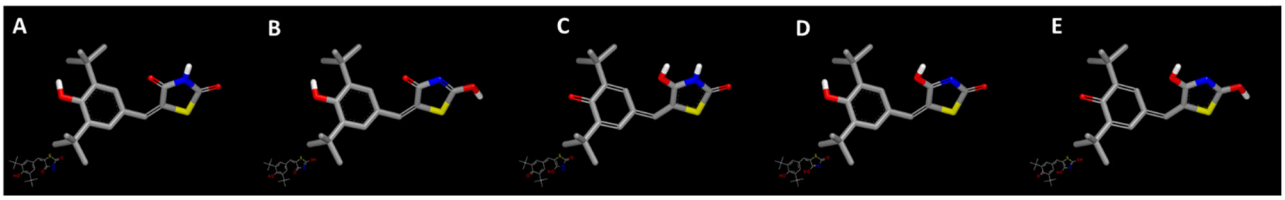

Fig. 5 Tautomers of CI-987. The tautomers reveal that CI-987 may form keto-enols in the warhead as well as a potential diol

potent, with an IC50 of 3.6 µM for galactose media, and 25.18 µM in high glucose media. We analyzed the potential tautomers in silico for both NL-1 (Fig. 4) and CI-987 (Fig. 5). The results from the computational study suggested that the TZD ring of NL-1 can form tautomers between the diketone and a keto–enol, whereas CI-987 was able to have additional di-enols possible. These tautomer differences could in part explain the activity differences between NL-1 and CI-987. To evaluate if this effect can be seen experimentally, we used different pH conditions to study the ability of CI-987 to display tautomerism as a function of pH (Fig. 6). We incubated CI-987 with different buffers and found that at pH 9, a strong yellow color shift was noticeable in the 96-well plate and seen in the colorimetric shift with the appearance of the 400–500 nm peak. The maximal absorption of CI-987 (lamda max) was found to be 350 nm, with a yellow color visible.

Structural comparison of the TZD NL-1 with the compound SCF-2, show that there was a marked loss of activity with the replacement of the TZD warhead with a rhodanine

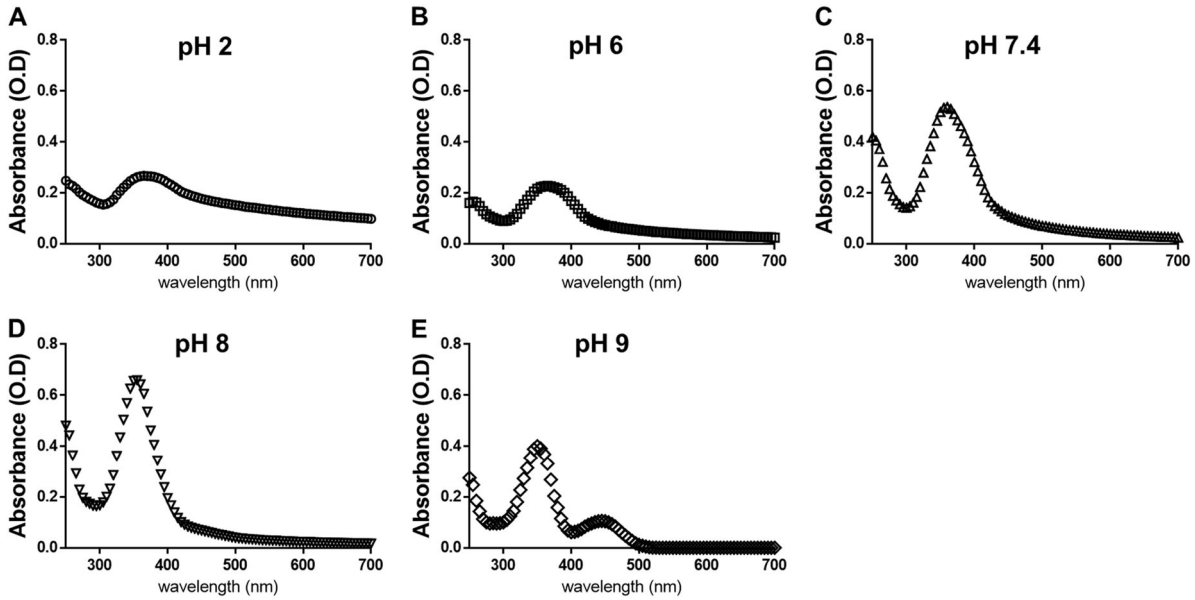

Fig. 6 Effect on tautomer with changes in pH. At pH of 9, the expected conjugated tautomer of CI-987 leads to the yellow color seen between 400–500 nm

moiety. This was surprising, due to such a small change in the structure. We theorized that the rhodanine warhead is more promiscuous which would likely reduce the effective intracellular concentration and therefore reduce the effective target engagement. Future studies will be designed to elucidate the targets associated with SCF-2 activity.

The tert-butyl groups of NL-1 seemed to be necessary for activity, since NL-2 showed marked reduction in antiproliferative activity in the MTT assay from 7.18 μM for NL-1 to 19.12 μM for NL-2. Compounds **10** and **11** provide some insight into the para position, in that the two compounds showed similar activities, (**10**: IC50 = 4.4 μM and **11**: IC50 = 4.8 μM), when a CF3 moiety was added instead of the hydroxyl of NL-1. This was also evident in **15** and **16**, with the replacement of the tert-butyl groups with bromines showed similar activity as the methyl of NL-2. For the TZDs the most promising leads from this study was found to be the benzopyran-4-one derivatives. These compounds inhibited cell proliferation with IC50s of 1.12 μM, indicating possible optimal positing of both steric, hydrophobic, and hydrogen boding interactions. These compounds will be used as template for future structural evaluation of side chain substitution.

Substitution of the amine from the TZD ring decreased the activity when comparing the antiproliferative activity to that of NL-1. For instance, epalrestat, an aldose reductase inhibitor which was readily available to test effect of TZD nitrogen substitution (Jaiswal et al. 2018), showed the lowest activity profile, with an IC50 of 407 μM. This trend was repeated with compound **3**, where the IC_{50} was 81 μM. These findings suggest that the attachment of a moiety to the amine wither sterically prevents the ketones from interaction with amino acids optimally.

In our phenotypic screen, we also included some five-membered rings systems as control compounds. As expected from this subset of compound there was a divergent activity profile. Compound **17** proved to be the most potent of this diverse class of compounds, with an IC_{50} of 0.98 μM. In terms of complexity, compounds **19** and **21** showed similar activity, with the addition of the di-Br substitution only marginally increasing activity of antiproliferation from an IC_{50} of 17.33 μM for compound **21** to an IC_{50} of 13.40 μM. Interestingly, compounds **19** and **21** are the derivatives of the antihistamine dimebon, which was also evaluated as potential anti-Alzheimer's disease therapy (Eckert et al. 2018). With new data emerging that indicates a possible mitochondrial activity profile, our assay was able to identify potential lead structures due to the use of galactose as fuel source.

Conclusion

Our phenotypic screen was based on identifying mitochondrial relevant hits, identified the use of the TZD scaffold as useful to develop novel leads. The use of galactose media enabled a mitochondrial-driven phenotypic screen with a cellular energy shift towards OXPHOS. The substitution pattern on the TZD warhead and the aromatic ring had pronounced effect on the antiproliferation activity. In addition, other five-membered rings were found to affect mitochondria, which allows for diversification in chemistry

programs. These compounds can be used to develop a new pipeline which targets specifically the differential metabolism of cancer cells.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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