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Mitotic arrest of breast cancer MDA-MB-231 cells by a halogenated thieno[3,2-*d*]pyrimidine

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Abstract

Halogenated thieno[3,2-*d*]pyrimidines exhibit antiproliferative activity against a variety of cancer cell models, such as the mouse lymphocytic leukemia cell line L1210 in which they induce apoptosis independent of cell cycle arrest. Here we assessed these activities on MDA-MB-231 cells, a well-established model of aggressive, metastatic breast cancer. While 2,4-dichloro[3,2-*d*]pyrimidine was less toxic to MDA-MB-231 cells than previously observed in the L1210 model, flow cytometry analysis showed that MDA-MB-231 cell death involved arrest at the G2/M stage of the cell cycle. Conversely, the introduction of bromine at C7 of the 2,4-dichloro[3,2-*d*]pyrimidine eliminated cell type-dependent differences in cytotoxicity or cell cycle status. Together, these data indicate that a substituent at C7 can profoundly modify the cytotoxic mechanism of halogenated thieno[3,2-*d*]pyrimidines in a cell type-specific manner.

Keywords

Thieno[3,2-*d*]pyrimidines; Triple negative breast cancer; Mitotic arrest; Cell cycle

Breast cancer is a major cause of cancer-related death in women preceded only by lung cancer.¹ The expression levels of estrogen receptors (ER) and progesterone receptors (PR) as well as the amplification status of the HER-2/Neu gene help direct diagnosis and treatment of breast cancer. For tumors that express one or more of these biomarkers, targeted therapies have significantly improved patient outcomes. However, breast cancers lacking the aforementioned biomarkers, termed triple negative breast cancer (TNBC), present severe challenges for patient survival since biomarker-targeted therapies are ineffective.¹

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Supplementary data

Supplementary data (materials and methods used for cytostatic assays, cell cycle distribution assays and analytical data (¹H NMR, ¹³C NMR and HRMS) for compound 1 and compound 2) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmcl.2015.02.071>.

Thienopyrimidine scaffolds have been extensively used in drug discovery and have found particular utility as inhibitors of cellular kinases in cancer treatment.²⁻⁵ Our interest in fused bicyclic pyrimidines led to findings that halogenated thieno[3,2-*d*]pyrimidines **1** and **2** were toxic to three different neoplastic cell lines at low to sub-micromolar concentrations (Fig. 1).⁶ The sulfur and chlorine at C6 (highlighted in red) were found to be critical to their cytotoxicity towards the tested cell models. Using the mouse lymphocytic leukemia cell line L1210 we further showed that both compounds induced apoptosis independent of cell cycle arrest. Continuing this work, we have now tested the cytotoxicity of these compounds on MDA-MB-231 cells, an extensively used cultured cell model of TNBC.^{1,7} MDA-MB-231 cells are also a popular model of metastatic breast cancer, as they were originally isolated from a pleural effusion and readily colonize at distal sites in mouse models.^{8,9} Since metastasis is responsible for the majority of breast cancer-related morbidity and mortality, continuing searches for new anti-neoplastic agents are critical if further improvements in breast cancer survival are to be achieved.¹⁰ Given this need for new treatment options targeting metastatic TNBC cells and the potent cytotoxicity displayed by compounds **1** and **2** against other cancer cell lines, in this study we tested their efficacy and cytotoxic mechanisms using the MDA-MB-231 cell model.

In our previous work, cell counting assays revealed potent cytotoxic effects of compounds **1** and **2** against L1210 cells, the human T lymphoblastic leukemia cell line CEM, and the human cervical adenocarcinoma cell line HeLa (Fig. 1). Curiously, compound **1**, which lacks the Br at C7 present in compound **2**, enhanced toxicity for L1210 cells by almost an order of magnitude, a finding validated using MTT assays,⁶ although it did not significantly alter its activity towards the other cell types. Here, MTT assays revealed that compounds **1** and **2** are both toxic to the TNBC cell model MDA-MB-231 at low-micromolar concentrations (Fig. 2). Analogous to findings with CEM and HeLa cells, cytotoxicity for MDA-MB-231 cells was not significantly impacted by the presence of Br at C7 in compound **2**.

In L1210 cells, compounds **1** and **2** both induced apoptosis but involving a mechanism independent of cell cycle arrest.⁶ To test whether the toxicity of these agents for MDA-MB-231 cells proceeded via a similar pathway, we monitored their effects on cell cycle distributions using propidium iodide staining and flow cytometry. Cumulatively over 80% of vehicle-treated MDA-MB-231 cells were confined to G1 and S phases, with much smaller subpopulations recovered in G2/M (10%) or in cells showing <G1 (<3%) or >G2 (<3%) DNA content (Fig. 3). Treating MDA-MB-231 cells with compound **2** had no effect on their cell cycle distribution, consistent with observations in the L1210 line. However, after treating MDA-MB-231 cells with compound **1**, the vast majority (>70%) accumulated in the G2/M phase of the cell cycle. Cell subpopulations in G1 and S phases were concomitantly depleted while no changes were observed in the fraction of cells with <G1 or >G2 DNA content. This dramatic accumulation of cells in G2/M indicates that compound **1**, but not compound **2**, triggers mitotic arrest in MDA-MB-231 cells. This distinction may also contribute to the differences in Hill slopes observed in cytotoxicity assays with these compounds (Fig. 2). Compound **1** consistently yielded a gentler Hill slope ($h = 1.22 \pm 0.32$) than compound **2** ($h = 2.18 \pm 0.15$, $P = 0.009$ vs compound **1**). Shallow drug response

curves may reflect enhanced cell-to-cell variability in drug sensitivity across the sample population,¹¹ which could indicate that MDA-MB-231 cells are differentially sensitive to compound **1** at different points in the cell cycle.

Many cytotoxic compounds function by activating cellular death pathways including apoptosis and necrosis, however, tumor cells typically acquire resistance to one or more of these pathways.^{12,13} In general, breast cancers tend to be resistant to therapeutic induction of apoptosis owing to frequent overexpression of select anti-apoptotic factors including Bcl-2 family members and the cyclin-dependent kinase inhibitor p21, as well as loss of the tumor suppressor p53.^{14,15} Weakened responsiveness of MDA-MB-231 cells to apoptotic stimuli includes contributions from elevated levels of the apoptosis inhibitor Bcl-x_L and a mutation in p53.¹⁶ Based on these findings, it has been proposed that therapeutic strategies promoting non-apoptotic cell death mechanisms may be more useful in treating breast cancers. Cell cycle arrest at G2/M can trigger an apoptosis-independent cell death pathway termed mitotic catastrophe, which in many cell types represents the primary mode of cell death following treatment with ionizing radiation or select chemotherapeutic agents.^{17,18} Pharmacologically, mitotic catastrophe can be induced by inhibiting the dynamic reorganization of microtubules (e.g., docetaxel, paclitaxel), resulting in activation of the mitotic spindle checkpoint. If this damage is not repaired, the check point eventually releases which in turn permits improper chromosome segregation leading to aberrant mitosis and ultimately necrosis-like cell death.¹⁹ However, beyond microtubule-targeting agents, some inhibitors of motor proteins and cellular kinases including those responsible for cell cycle progression (e.g., CDK1) and centrosome maturation and separation (e.g., Aurora kinase and polo-like kinase (Plk) families) can also induce mitotic catastrophe.²⁰

In this work we show that both compounds **1** and **2** are toxic to MDA-MB-231 cells, but that only for compound **1** is this associated with mitotic arrest, a novel finding that we did not observe in other cell models tested.⁶ Given the known roles of select thienopyrimidine scaffold drugs as kinase inhibitors,^{2–5} it is appealing to hypothesize that a critical cell cycle or mitotic kinase may be targeted by compound **1**. However, equally intriguing is the observation that addition of bromine at C7 in compound **2** abrogates the mitotic consequences of this scaffold without significantly attenuating overall cytotoxicity, indicating that these compounds function through distinct molecular targets in the MDA-MB-231 line. The fact that this subtle substitution on the thienopyrimidine scaffold can elicit such a profound change in the cytotoxic mechanism raises the exciting possibility that similar modifications may allow further fine-tuning of cell toxicity and/or selectivity for specific cell types, tumor stages, or other physiological distinctions. Finally, the demonstration that compound **1** can selectively activate mitotic arrest in the breast cancer cell model may be particularly significant given the need for novel compounds targeting this mechanism as lead candidates for new breast cancer treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Antiproliferative activity of halogenated thieno[3,2-*d*]pyrimidines against indicated cancer cell lines. IC₅₀ values are quoted from Ref. 6.

Figure 2.

MTT assays of cytotoxicity by **1** and **2** in MDA-MB-231 cells. Cells were treated with selected concentrations of compounds **1** (left) and **2** (right). After 48 h, cell viability was measured using MTT assays. Drug concentrations yielding 50% cell death (IC_{50}) were calculated by non-linear regression to a sigmoidal dose response function and are quoted as the mean $\pm$ SD of three independent experiments.

Figure 3.

Cell cycle analyses of MDA-MB-231 cells following treatment with **1** and **2**. Cell cycle distributions of MDA-MB-231 cells after 48 h in the presence of equitoxic (IC_{80}) doses of compound **1** (15 μ M) or compound **2** (8 μ M) analyzed by flow cytometry of fixed, propidium iodide-stained cells and compared to vehicle controls (DMSO). A minimum of 3000 cells were analyzed per cell population. Each bar represents the mean $\pm$ SD across 4 independent cell samples.