INTRODUCTION

Cancer universally claims the lives of over 9.5 million individuals each year.1,2 Breast cancer is of particular concern as it is the most common cause of cancer fatalities in women worldwide.3 Approximately 30% of breast cancers metastasize, a process in which the cancer spreads to other body regions. Metastatic breast cancers respond poorly to current treatment options and the prognosis for patients is dire, with a 5-year mortality rate of over 70%.3 Metastatic breast cancers are especially difficult to treat as they are often multi-drug resistant (MDR) and have little to no expression of estrogen receptors (ER) and progesterone receptors (PR), and lack overexpression of human epidermal growth factor receptors 2; HER2.

Abstract

Breast cancer causes the most cancer fatalities in women worldwide. Approximately one-third of breast cancers metastasize, or spread from primary tumors to other tissues, and have a 70% 5-year mortality rate. Current breast cancer treatments like doxorubicin and paclitaxel become ineffective when breast cancer cells develop multi-drug resistance and overexpress ATP-binding cassette transporters, as the transporters cause a substantial efflux of the chemotherapies. Jadomycins, a group of molecules isolated from Streptomyces venezuelae ISP5230, are shown to be cytotoxic against a variety of cancers, especially breast cancer. Furthermore, jadomycins retain their cytotoxic properties in multi-drug resistant breast cancer cells, as they are not expelled through ATP-binding cassette transporters. Here, we describe the research that supports the potential use of jadomycins as a novel chemotherapy in the treatment of multi-drug resistant, metastatic breast cancer. We present the supportive findings, as well as the mechanisms of action investigated thus far. These include copper-mediated reactive oxygen species generation, aurora B kinase inhibition, and topoisomerase IIα and IIβ inhibition. We also suggest future directions of jadomycin research, which will help to determine if jadomycins can be used as a breast cancer chemotherapy in clinical practice.

Keywords

chemotherapy, jadomycins, metastatic breast cancer, multi-drug resistance
receptor 2 (HER2). These receptors are key breast cancer drug targets and serve as diagnostics to identify specific types and stages of breast cancer.4 When breast cancers lack all three of these receptors, they are known as triple-negative breast cancer. There are currently few effective treatments for MDR metastatic breast cancers, and despite treatment breast cancer cells often continue to proliferate and gain resistance to most available chemotherapies, which become ineffective.5 Thus, research for new MDR metastatic breast cancer therapies is imperative.

Jadomycins, a group of molecules first isolated in 1991 from the soil bacteria Streptomyces venezuelae ISP5230, have demonstrated promising cytotoxicity against triple-negative and receptor positive MDR metastatic breast cancer cells, and may therefore be useful as a novel chemotherapy.6–10 Here, we review the current evidence pertaining to jadomycin cytotoxicity in various cancer cell lines, with a specific focus on MDR breast cancer. Our search strategy utilized Google Scholar and the PubMed and Novanet databases, and included the key terms "jadomycin," "copper dependency," "reactive oxygen species," "aurora B kinase," and "topoisomerase." We explain the present knowledge of jadomycin cytotoxic mechanisms in MDR breast cancer cells, including their copper-dependent reactive oxygen species (ROS) generation and aurora B kinase (ABK), and topoisomerase II interactions.7,8,11,12 We also discuss future directions for the study of jadomycins, including the need to investigate jadomycin anticancer effects in animal models, and to further define jadomycin mechanism of action to predict possible adverse drug effects and drug-drug interactions.

2 JADOMYCIN BIOSYNTHESIS

Jadomycins belong to the angucycline, type-II polyketide-derived family of molecules, and are produced by S. venezuelae under stress conditions of ethanol treatment, phage infection, or heat shock (Figure 1A).10,12 S. venezuelae is known for its production of the antibiotic chloramphenicol, and jadomycins exhibit similar antimicrobial activity against both Gram positive and negative bacteria (Figure 1B).13,14 Additionally, jadomycins demonstrate anticancer properties against hepatic, lung, multiple myeloma, cervical, colon, and breast cancer cell lines.7–9,11,15–17 For a more complete description of jadomycin biosynthesis we refer the interested reader to the recent review article by de Koning et al.18

While most jadomycins maintain the same polyaromatic core structure, usually consisting of an A-, B-, C-, D-, and E-ring, their various amino acid side groups make them unique (Figure 2A).10 S. venezuelae grown in media containing a single amino acid as the nitrogen source selectively produces diverse analogs of the jadomycin molecule.10 Interestingly, S. venezuelae incorporates amino acids into the jadomycin polyaromatic core through chemical biosynthesis in a non-enzymatic manner.10,19 This is an important feature, as over 70 jadomycins are conveniently synthesized in vitro through chemical methods, which facilitates structural diversity around the E-ring and produces jadomycins with unique pharmacological properties (Figure 2A).10 However, the ability to synthesize jadomycin derivatives through the use of selective media is not always achieved in the presence of a single amino acid.20 For example, media containing 3- or 4-(aminomethyl)benzoic acid (3AMBA and 4AMBA) normally inhibits S. venezuelae proliferation, but the addition of D-serine to the media allows the bacteria to add 3AMBA or 4AMBA acid to the jadomycin backbone.21 This suggests that D-serine promotes the incorporation of other products to create novel jadomycins, which could further enhance jadomycin pharmacological diversity.21

Jadomycin A was the first to be discovered and is structurally identical to jadomycin B, but contains a hydroxyl group on the D-ring instead of a deoxy sugar (Figure 2A).10 Jadomycin A and B were named in order of discovery, but subsequent jadomycins were named based on their R2 side groups (Table 1). Most jadomycins exhibit some pharmacological significance, but jadomycin A and W in particular have little cytotoxic activity against bacteria and cancer cells, attributed to the lack of a glycone ring and a large aromatic structure, respectively.9,15,16 Large amino acids on the E-ring generally prevent jadomycins from interacting precisely with cellular targets. However, there is additional versatility in the E-ring, as the R2 attachment is not strictly limited to amino acids. Jadomycin N, for example, incorporates a carboxyl group in this location, yet still possesses anticancer properties.16 Notably, various enzymes can also catalyze prejadomycin, from which jadomycin A is derived, into alternative natural products that may possess additional benefits.22

Jadomycins are believed to maintain their cytotoxic properties in MDR breast cancer cells because unlike standard metastatic breast cancer chemotherapies including taxanes, anthracyclines, and epothilones, many of the most potent jadomycins exhibit an oxazolone or oxazolidine ring side chain which may result in unique interactions with cancer cells (Figure 2B–D).9,16 Nonetheless, researchers have noticed that jadomycins with different chemical structures can also exhibit anticancer properties. For instance, jadomycins with a 3AMBA side group lack an E-ring, but are still cytotoxic against breast cancer cells.21 Jadomycins with a triazole group attached to the E-ring are also cytotoxic against various cancer cells, while those with a non-triazole group attachment demonstrate little to no cytotoxic activity.23 This argues that while the A, B, C, and D-rings are involved in the anticancer effect of jadomycins, the glycone ring attachment on the D-ring and structural diversity around the E-ring are most involved in changing the potency and efficacy of jadomycins.

Several experiments have shown that jadomycins have the unique ability to avoid ATP-binding cassette (ABC) drug efflux transporters. ABC transporters are pumps that expel intracellular drugs and toxins, and are often overexpressed in MDR breast cancer cells, which may contribute to the avoidance of cell death.7,24,25 Jadomycins have demonstrated the ability to maintain their potency and efficacy in both drug-sensitive and drug-resistant breast cancer cells, unlike the structurally similar doxorubicin (DOX) and mitoxantrone (MITX) chemotherapies used to treat triple-negative breast cancer (Figure 1C and D). Thus, jadomycins are a potentially attractive chemotherapeutic alternative, and may contribute to an increasingly important area of cancer research.7
JADOMYCINS ARE CYTOTOXIC TO BREAST CANCER CELL LINES

Several studies have verified that jadomycins exhibit anticancer properties in multiple human breast cancer cell lines. The evidence supporting jadomycin cytotoxicity against each breast cancer type is highlighted in the following sections.

3.1 | Hormone-receptor positive breast cancer

3.1.1 | T-47D cells

Borissow et al. were among the first to explore jadomycin cytotoxicity in breast cancer cells. They assessed cytotoxicity profiles of jadomycin βala, B, DM, DT, DV, F, G, H, M, N, R-Phe, S, SPG, T, V, W, and Y against the ER positive (+), PR+, and HER2 negative (−) human breast cancer cell line T-47D. The results indicated that jadomycin DT and S were the most potent overall, while jadomycin βala, H, and Y were among the least potent (Table 2). Jadomycin DT and S both have polar carboxy side chains, which may be responsible for their ability to induce cancer cell death. Polarity of the side chain is perhaps more important than size, as jadomycin G has one of the smallest side chains but is non-polar and exhibits little pharmacological activity. Aromatic amino acids also appear to sterically hinder jadomycin interaction with cancer cells, as jadomycin H and Y with histidine and tyrosine groups, respectively, were less efficacious than jadomycins with nonaromatic groups.
3.1.2 | MCF7 cells

Most studies on jadomycin cytotoxicity against breast cancer have used the human metastatic, ER+/PR+/HER2- breast cancer cell line MCF7, as it is an ideal model for the study of anticancer drug mechanisms and is widely accessible. Fu et al. used thiazolyl blue methyltetrazolium bromide (MTT) colorimetric assays to quantify jadomycin cytotoxicity. The MTTs determined that jadomycin B, S, and T half maximal inhibitory concentration (IC₅₀) values, the concentration of drug that inhibits 50% of cell growth, ranged from 23.3 to 29.4 µM and produced cytotoxic effects in MCF7 cells (Table 2). In comparison, Fan et al. assessed the cytotoxicity of jadomycin Abu, B, Hse, L, Nle, Orn, S, T, and V in MCF7 cells using sulforhodamine B (SRB) assays, which measure cell density. Their results revealed that while jadomycin T had the lowest IC₅₀, jadomycin Abu, B, L, and V all shared similar values (Table 2).

Issa et al. revisited the interactions between jadomycins and MCF7 cells using jadomycin B, DNV, F, L, S, SPH, and T and W in MCF7 control (MCF7-CON), paclitaxel-resistant (MCF7-TXL), etoposide-resistant (MCF7-ETP), and MITX-resistant (MCF7-MITX) cells. A corresponding increase of mRNA verified that the MCF7-TXL, MCF7-ETP, and MCF7-MITX cells overexpressed ABCB1, ABCC1, and ABCG2 transporters, respectively. With IC₅₀ ranging from 1.3 to 4.4 µM, jadomycin B, DNV, F, L, S, SPH, and T were all able to reduce MCF7-CON cell viability to approximately 0%, while jadomycin W had a higher IC₅₀ of 19 µM and only reduced cell viability to approximately 20% (Table 2). These findings indicate that jadomycin W, which contains a large aromatic side group, has stereochemical hinderance and limited interaction in cancer cells. All jadomycins tested also reduced cell viability in MCF7-TXL, MCF7-ETP, and MCF7-MITX cells with a small decrease in potency, suggesting that their anticancer properties are largely retained in ABC-overexpressing MDR breast cancer.

3.1.3 | BT474 and SKBR3 cells

Jadomycin cytotoxicity was tested in the human ER+/PR+/HER2+ BT474 and ER+/PR+/HER2+ SKBR3 breast cancer cells lines, and the results were compared to MCF7-CON and triple-negative ER-/PR-/HER2- MDA-MB-231 breast cancer cells. Jadomycin B, F, and S had similar cytotoxicity in BT474, SKBR3, MDA-MB-231, and MCF7-CON cell lines, with slightly varying potencies (Table 2). These findings indicate that jadomycin cytotoxicity is likely independent of hormone receptor status.
3.2 | Triple-negative breast cancer

3.2.1 | MDA-MB-435 cells

Borissow et al. tested jadomycin βala, B, DM, DT, DV, F, G, H, M, N, R-Phe, S, SPhG, T, V, W, and Y against ER-/PR-/HER2- MDA-MB-435 cancer cells, and the results were very similar to those observed in T-47D cells (Table 2).\(^\text{16}\) Jadomycin DT and S were most potent against MDA-MB-435, while jadomycin βala, H, and Y were least potent. Most jadomycins demonstrated greater potency against MDA-MB-435 than T-47D, but jadomycin H was two-fold more potent in T-47D cells, suggesting it may interact differently in hormone receptor positive breast cancer.\(^\text{16}\) However, some doubt surrounds the authenticity of the MDA-MB-435 cell line. At the time of publication, it was believed that MDA-MB-435 was a triple-negative breast cancer cell line, but it has since been proposed that MDA-MB-435 cell samples derive from a melanoma cell line.\(^\text{29-31}\) As such, these results may give insight into jadomycin cytotoxicity against melanoma, but it is necessary to repeat the experiments in authentic melanoma cells to verify this presumption.

3.2.2 | MDA-MB-231 cells

Hall et al. continued to explore jadomycin avoidance of ABC transporters in triple-negative MDA-MB-231 control (231-CON) and MDA-MB-231 paclitaxel-resistant (231-TXL) breast cancer cells.\(^\text{7}\) As expected, triple-negative breast cancer chemotherapies DOX and MITX were effective against 231-CON cells (Figure 3A) but were significantly less potent in 231-TXL cells (Figure 3B).\(^\text{7}\) Jadomycins, however, retained their cytotoxic potency in 231-TXL cells, despite the overexpression of ABCB1 transporters (Table 2; Figure 3B).\(^\text{7}\) These findings indicate that jadomycins may be useful as a chemotherapy for MDR breast cancer regardless of ER, PR, or HER2 status. Additionally, this supports that jadomycins should be explored as an alternative or additive to current breast cancer chemotherapies as they retain their efficacy in ABC-overexpressing breast cancer cells.\(^\text{7,9}\)

4 | JADOMYCIN MECHANISMS OF ACTION

Understanding the mechanism(s) of jadomycins is necessary to determine their clinical usefulness against specific types of breast cancer and to predict adverse drug effects and drug–drug interactions. The following sections summarize previously proposed jadomycin mechanisms of action, and the current evidence supporting jadomycin polypharmacology.

4.1 | APOPTOSIS

Apoptosis is a process that controls cell death to maintain homeostasis in body tissues and is defective in many cancers through up- or downregulation of various genes that result in the unchecked proliferation of cancer cells.\(^\text{32-34}\) Evidence from earlier studies of HepG2 hepatic cancer cells, A549 non-small cell lung carcinoma (NSCLC) cells, and IM-9 myeloma cells supports that jadomycins activate apoptosis as a mechanism of cancer cell death.\(^\text{15,15}\) Jadomycin cytotoxicity was also tested in the Bcl-2 overexpressing myeloma sub-line IM-9/Bcl-2. Bcl-2 is a protein that regulates apoptosis, and overexpression of Bcl-2 leads to resistance to standard chemotherapies. Jadomycins were able to overcome Bcl-2 overexpression, albeit at exceptionally high doses as the cells demonstrated more than two-fold resistance.\(^\text{15}\)
| Study             | Cell type | Cell line | Exposure time (h) | Assay used | IC₅₀ (µM) | βala | Abu | Ala | B | DM | DNV | DT | DV | F | G | H | Hse |
|------------------|-----------|-----------|------------------|------------|-----------|------|-----|-----|---|----|-----|----|----|---|---|---|-----|
| Borissow 2007    | Breast    | T-47D     | 48               | MTT        | 23.11     | 6.70 | 8.16 | 5.16 | 5.16 | 2.90 | 7.57 | 4.04 | 7.44 | 9.03 | 31.07 |
| Fu 2008          | Breast    | MCF7-CON  | 48               | MTT        | 27.2      | 10.0  | 10.0 | 23.3 | 23.3 | 29.4  | 7.13 | 19.3  | 9.5  | 21.5 | 30.0 |
| Fan 2012         | Breast    | MCF7-CON  | 72               | SRB        | 9.9       | 4.0   | 4.0  | 5.5  | 5.5  | 4.0   | 1.9   | 2.8   | 2.5  | 19.3  | 19.3 |
| Issa 2014        | Breast    | MCF7-CON  | 72               | MTT        | 4.4       | 1.3   | 1.3  | 3.4  | 3.4  | 1.9   | 2.8   | 2.5   | 19.3  | 19.3 |
| Hall 2015        | Breast    | MCF7-CON  | 72               | MTT        | 2.6       | 1.0   | 1.0  | 3.1  | 3.1  | 3.1   | 3.1   | 3.1   | 3.1   | 3.1   |
| Issa 2014        | Breast    | MCF7-TXL  | 72               | MTT        | 2.8       | 2.8   | 2.8  | 3.6  | 3.6  | 3.6   | 3.6   | 3.6   | 3.6   | 3.6   |
| Issa 2014        | Breast    | MCF7-ETP  | 72               | MTT        | 8.0       | 2.2   | 2.2  | 3.2  | 3.2  | 3.2   | 3.2   | 3.2   | 3.2   | 3.2   |
| Issa 2014        | Breast    | MCF7-MITX | 72               | MTT        | 9.9       | 4.2   | 4.2  | 3.6  | 3.6  | 3.6   | 3.6   | 3.6   | 3.6   | 3.6   |
| Hall 2015        | Breast    | BT474     | 72               | MTT        | 9.9       | 4.2   | 4.2  | 3.6  | 3.6  | 3.6   | 3.6   | 3.6   | 3.6   | 3.6   |
| Hall 2015        | Breast    | SKBR3     | 72               | MTT        | 3.8       | 1.0   | 1.0  | 3.6  | 3.6  | 3.6   | 3.6   | 3.6   | 3.6   | 3.6   |
| Borissow 2007    | Breast    | MDA-MB-435| 48               | MTT        | 10.6      | 6.89  | 6.89 | 1.15 | 1.15 | 3.24  | 6.11  | 3.35  | 3.35  | 20.99 |
| Hall 2015        | Breast    | 231-CON   | 72               | MTT        | 1.8       | 1.8   | 1.8  | 3.3  | 3.3  | 3.3   | 3.3   | 3.3   | 3.3   | 3.3   |
| Hall 2015        | Breast    | 231-CON   | 72               | MTT        | 2.8       | 2.8   | 2.8  | 3.0  | 3.0  | 3.0   | 3.0   | 3.0   | 3.0   | 3.0   |
| Hall 2015        | Breast    | 231-TXL   | 72               | MTT        | 2.5       | 2.5   | 2.5  | 3.2  | 3.2  | 3.2   | 3.2   | 3.2   | 3.2   | 3.2   |
| Zheng 2005       | Hepatic   | HepG2     | 24               | MTT        | 100.0     | 10.8  | 10.8 | 49.0 | 49.0 | 49.0  | 49.0  | 49.0  | 49.0  | 49.0  |
| Zheng 2005       | Myeloma   | IM9       | 24               | MTS        | 40.0      | 8.5   | 8.5  | 29.0 | 29.0 | 29.0  | 29.0  | 29.0  | 29.0  | 29.0  |
| Zheng 2005       | Lung      | H460      | 24               | MTT        | 30.7      | 21.8  | 21.8 | 12.4 | 12.4 | 12.4  | 12.4  | 12.4  | 12.4  | 12.4  |
| Fu 2008          | Lung      | A549      | 48               | MTT        | 11.3      | 11.3  | 11.3 | 11.3 | 11.3 | 11.3  | 11.3  | 11.3  | 11.3  | 11.3  |
| Fu 2008          | Cervix    | HeLa      | 48               | MTT        | 18.2      | 18.2  | 18.2 | 18.2 | 18.2 | 18.2  | 18.2  | 18.2  | 18.2  | 18.2  |
| Fan 2012         | Colon     | HCT116    | 72               | SRB        | 8.3       | 3.8   | 3.8  | 56.8 | 56.8 | 56.8  | 56.8  | 56.8  | 56.8  | 56.8  |
| Fan 2012         | Epithelium| HMEC      | 72               | SRB        | 3.0       | 2.3   | 2.3  | 62.9 | 62.9 | 62.9  | 62.9  | 62.9  | 62.9  | 62.9  | 62.9  |

(Continues)
| Study          | Cell type | Cell line | Exposure time (h) | Assay used | IC50 (µM) | L | M | N | Nle | Om | R-Phe | S | SPhG | T | V | W | Y |
|---------------|-----------|-----------|------------------|------------|-----------|---|---|---|-----|----|-------|---|-----|---|---|---|---|
| Hall 20158    | Breast    | BT474     | 72               | MTT        | —          | — | — | — | —   | —  | —     | 3.1| —   | — | — | — | — |
| Hall 20158    | Breast    | SKBR3     | 72               | MTT        | —          | — | — | — | —   | —  | —     | 3.1| —   | — | — | — | — |
| Borissow 2007 | Breast    | MDA-MB-435| 48               | MTT        | —          | 3.57| 4.01| — | —   | 1.57 | 1.06 | 3.50| 2.82 | 3.76| 7.60| 10.65|
| Hall 20158    | Breast    | 231-CON   | 72               | MTT        | —          | — | — | — | —   | —  | —     | 2.8| —   | — | — | — | — |
| Hall 20177    | Breast    | 231-TXL   | 72               | MTT        | —          | — | — | — | —   | —  | —     | 2.8| —   | — | — | — | — |
| Hall 20177    | Breast    | 231-CON   | 72               | MTT        | —          | — | — | — | —   | —  | —     | 2.8| —   | — | — | — | — |
| Hall 20177    | Breast    | 231-CON   | 72               | MTT        | —          | — | — | — | —   | —  | —     | 2.8| —   | — | — | — | — |
| Zheng 200515  | Hepatic   | HepG2     | 24               | MTT        | —          | — | — | — | —   | —  | —     | 9.8| 27.0| 27.0| — | — | — |
| Zheng 200515  | Myeloma   | IM9       | 24               | MTS        | —          | — | — | — | —   | —  | —     | 6.3| 9.1 | 8.2| — | — | — |
| Zheng 200515  | Lung      | H460      | 24               | MTT        | —          | — | — | — | —   | —  | —     | 19.2| 19.6| — | — | — | — |
| Fu 200811     | Lung      | A549      | 48               | MTT        | —          | — | — | — | —   | —  | —     | 38.4| 47.8| — | — | — | — |
| Fu 200811     | Cervix    | HeLa      | 48               | MTT        | —          | — | — | — | —   | —  | 24.5   | 26.7| —   | — | — | — | — |
| Fan 201217    | Colon     | HCT116    | 72               | SRB        | 3.8        | — | — | 14.5| 5.4 | 4.6 | —     | 1.3| 4.4 | — | — | — | — |
| Fan 201217    | Epithelium| HMEC      | 72               | SRB        | 2.1        | — | — | 9.2 | 10.2| 4.3 | —     | 1.4| 2.0 | — | — | — | — |

Note: Jadomycin IC50 values (µM) determined by MTS, MTT, or SRB assays are compared across studies by cell line. Highlighted values are the lowest jadomycin IC50 values and generally most potent jadomycin derivative for the indicated cell line, as determined by the specified study. IC50, half maximal inhibitory concentration; MTS, phenazine methosulfate; MTT, thiazolyl blue methyltetrazolium bromide; SRB, sulforhodamine B.
Conclusively, apoptosis is also indicated as the main jadomycin-induced cell death process in breast cancer cells. One study showed that jadomycins B, S, and T caused apoptosis in MCF7 cells.\textsuperscript{11} Annexin V/PI staining indicated that jadomycins B, F, and S also caused apoptosis in 231-CON and 231-TXL cells.\textsuperscript{7} While jadomycins are also cytotoxic against NSCLC H460, cervical HeLa, and colon HCT116 cancer cells, it is unclear if they induce cell death through apoptosis in these cells. Hence, the most basic mechanistic details of jadomycin activity in lung, cervical, and colon cancers remains largely unknown and requires further investigation. Finally, while jadomycins are repeatedly shown to induce cell death through apoptosis, other non-apoptotic mechanisms of cell death are possible, including mitotic catastrophe or senescence, and may provide more insight into jadomycin cytotoxicity.\textsuperscript{35}

4.2 CU(II)-DEPENDANT ROS

Once jadomycin-induced apoptosis was observed in several cancer cell lines, investigation of the events leading to apoptosis, such as ROS induction, became a primary focus. ROS are highly reactive molecules that contain oxygen and often accumulate in cancer cells, leading to cancer progression and metastasis.\textsuperscript{34,37} However, chemotherapeutic drugs often induce additional ROS production in cancer cells and cause an accumulation of ROS beyond the tolerable threshold, resulting in cell toxicity and death.\textsuperscript{3,37} Thus, depending on the intracellular level, ROS can be either carcinogenic or anticarcinogenic.\textsuperscript{36,37} In the presence of copper, jadomycins have been shown to increase ROS production and the rate of cell death.\textsuperscript{8} Breast cancer cells often have increased intracellular levels of copper compared to normal breast tissue, which may facilitate the higher potency of jadomycins observed in breast cancer cells compared to other cell lines.\textsuperscript{38}

Researchers have explored the jadomycin-copper interaction that may propagate chemotherapeutic benefits. In extracellular bacterial supercoiled plasmid models, jadomycin B reduced Cu(II) to Cu(I) and resulted in DNA strand scission through the production of hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals.\textsuperscript{12} When 100 mM of copper chelator EDTA was added to the jadomycin B treatment no DNA damage was observed, suggesting that the presence of copper ions is necessary for jadomycin-induced DNA cleavage.\textsuperscript{12} H$_2$O$_2$ is also a necessity as treatment with catalase, a catalytic enzyme that reduces H$_2$O$_2$, resulted in total inhibition of DNA scission.\textsuperscript{12} Still, it is important to note that these plasmids contained high guanine-cytosine content and the results of this study may be slightly exaggerated since guanine is more susceptible to oxidative stress, which can cause DNA strand breaks.\textsuperscript{12,39}

Though the studies Monro et al. performed were impressive, they were limited to the observation of ROS effects in non-cellular assays.\textsuperscript{12} Therefore, it was important to determine if jadomycins would also increase ROS in breast cancer cells and lead to cytotoxicity. CM-DCHF$_2$-DA assays, used to measure ROS production in cells, revealed that CuSO$_4$ cotreated with jadomycin B, F, S, or SPHg in MCF7 cells increased ROS production and decreased cell viability.\textsuperscript{8} CuSO$_4$ treatment alone increased ROS but produced no cytotoxic effects, confirming an interaction between Cu and jadomycins is required for the observed increase in cell death.\textsuperscript{8} Furthermore, the Cu(II)-chelator D-penicillamine cotreated with jadomycin B, F, S, or SPHg in MCF7 cells reduced ROS production and jadomycin cytotoxicity, supporting the hypothesis that copper is either required for or supports the enhancement of jadomycin cytotoxicity.\textsuperscript{8}

To further investigate jadomycin ROS production and dependency, Hall et al. cotreated cells with antioxidants or inhibitors of cellular antioxidant pathways.\textsuperscript{8} When MCF7 cells were cotreated with antioxidant N-acetyl cysteine (NAC) and jadomycin B, F, S, or SPHg,
decreased ROS production and cytotoxic potency was observed for each jadomycin. NAC treatment affected the cytotoxicity of jadomycin B, F, and S more than jadomycin SPhG, suggesting that ROS-mediated toxicity depends on the specific jadomycin. Overall, these results indicated that jadomycin cytotoxicity is reduced when ROS production is hindered. However, increased doses of jadomycin B, F, S, or SPhG were still able to reduce the number of viable cells to less than 5%, even in the presence of NAC. It is possible that higher doses of jadomycin exceed the protective effect of NAC or stimulate a ROS-independent cytotoxic mechanism.

When MCF7-CON cells were cotreated with jadomycin B, F, S, or SPhG and auranofin, a prooxidant thioredoxin reductase (TrxR) inhibitor, ROS activity and jadomycin cytotoxicity significantly increased. Similar results were observed when the cells were cotreated with sodium diethyldithiocarbamate (DDC), a superoxide dismutase I inhibitor that blocks conversion of superoxide to H2O2. However, when MCF7 cells were cotreated with jadomycin and DDC, a superoxide dismutase II inhibitor, there was no change in ROS activity or cell viability. This may indicate that jadomycins increase ROS concentrations in the cytosol of MCF7 cells rather than in the mitochondria, the usual location of superoxide dismutase II. Therefore, jadomycins might have an advantage over DOX, which has an affinity for mitochondria-rich heart cells and causes cardiotoxicity. Interestingly, the glutathione S-transferase inhibitor ellagic acid (EA) did not affect ROS activity but still enhanced the cytotoxicity of jadomycin S and SPhG, again suggesting that some aspects of jadomycin cytotoxicity may be ROS-independent. Also, in the presence of jadomycin B, MCF7-CON cells significantly increased expression of the antioxidant gene TrxR1. This may be a cellular response to increased ROS, and lead to the activation of the Prx/Trx pathway to remove ROS and enhance survival. These findings are consistent with the earlier discussed experiment involving increased ROS activity during cotreatment with the TrxR inhibitor auranofin and jadomycin B.

When CM-DCHF$_2$-DA assays confirmed that jadomycins increase intracellular ROS activity in both 231-CON and 231-TXL cells, Hall et al. investigated if accumulation of double-strand DNA (dsDNA) breaks occurred as a downstream result of ROS activation. To do this Hall et al. measured nucleus-contained phosphorylated histone H2AX (γh2AX) protein using western blots to identify dsDNA breaks in both 231-CON and 231-TXL cells. Jadomycin B, F, S, and MITX all increased γh2AX protein expression in 231-CON cells. As a representative jadomycin, jadomycin S significantly increased γh2AX protein expression in 231-TXL cells while MITX lost its effect, a finding consistent with MITX being an ABCB1 substrate and jadomycins avoiding efflux transporters. Initially, most of these results appeared to indicate that jadomycins primarily cause dsDNA breaks and apoptosis through ROS-dependent pathways. However, when jadomycin F or S was cotreated with NAC, ROS decreased significantly in 231-CON cells, but there was no change in dsDNA damage. Likewise, when jadomycin F or S was cotreated with auranofin, ROS increased significantly but did not affect dsDNA damage. These results suggest that while copper-dependent ROS production augments jadomycin cytotoxicity, it is not the primary driving factor. It is also possible that such results are specific to certain breast cancer cell types.

### 4.3 ABK INHIBITION

While jadomycin-induction of ROS was being explored, jadomycins were also found to inhibit genes and proteins like ABK, which participates in chromatid separation and cytokinesis in eukaryotic mitosis. Inhibition of ABK causes cells to cease rapid proliferation. Furthermore, overexpression of ABK is common in hepatic, lung, and breast cancers, leading to increased proliferation. Hence, ABK may be an ideal anticancer target.

In 2008, Fu et al. investigated jadomycin B cytotoxic mechanisms in budding yeast cells containing the Ipl1 gene that encodes for Ipl1 aurora kinase, an analog of human ABK. Since the amino acid residues surrounding the ATP-binding site of Ipl1 kinase and ABK are nearly identical, the binding pocket where the ABK-competitive inhibitor hesperadin interaction was modeled and used as a virtual screening tool, which identified jadomycin B as a potential ABK inhibitor. Supporting the molecular docking prediction, jadomycin B was shown to competitively inhibit purified ABK in the presence of various concentrations of ATP. Two yeast strains were then used to compare the activity of potential ABK inhibitors: wild-type yeast, and ipl1-321 temperature-sensitive yeast, the latter of which expresses compromised Ipl1 kinase activity and was expected to have increased sensitivity to ABK inhibitors. Both strains were treated with 10 μg/ml jadomycin B, which decreased the percentage of ipl1-321 mutant growth to approximately 20% but had little to no effect on wild-type growth. Interestingly, jadomycin S and T demonstrated no growth inhibition against each yeast strain, and did not inhibit purified ABK, reinforcing the idea that jadomycin structure dictates its pharmacological interactions with ABK.

Although these findings initially supported the idea that jadomycin B acts as an ABK inhibitor, there were several outstanding questions. First, much of the research was conducted based on the assumption that Ipl1 kinase and ABK are homologues. While this is true, the researchers reached this conclusion based on the small ATP-binding site amino acid sequence rather than protein homology analysis, as the crystal structure of Ipl1 kinase was unavailable. Whereas it was acknowledged that jadomycin B may interact with other aurora kinases, the possibility of jadomycin B interacting with other non-mitotic proteins that could lead to downstream cascades and inhibit ABK was not considered. The anticancer effects of jadomycin S and T also appeared to occur through a pathway outside of ABK inhibition and suggested that other cytotoxic mechanisms of action must exist. Nonetheless, the overall results of jadomycin B as a potential ABK inhibitor were impressive and became a viable area of research.

Since ABK is also known to phosphorylate histone H3 within cells, Fu et al. used western blots to compare the amount of phosphorylated H3 (pHis3) in untreated versus jadomycin B-treated
While the untreated cells showed a clear gel band indicating pH3S, pH3S was undetectable in cells treated with 10 μg/ml of jadomycin B, indirectly demonstrating that jadomycin B blocks ABK activity. A dose-dependent decrease in pH3S was also observed in jadomycin B-treated hepatic HepG2 and cervical HeLa cells.11 Later, Issa et al. revealed that jadomycin B similarly inhibited pH3S in both MCF7-CON and MCF7-TXL cells in a dose-dependent manner, with a slightly reduced effect in MCF7-MITX cells.9 This finding suggested that the overexpression of ABCB1 and ABCG2 transporters does not interfere with ABK inhibition.9 Further research demonstrated that ABK inhibition proceeds through a ROS-independent mechanism, as jadomycin B-mediated reduction in pH3S and cytotoxicity was retained when ROS were inhibited, though with decreased potency.8,9 While there is evidence supporting direct jadomycin B inhibition of ABK, this may not be the primary mechanism that causes dsDNA damage. DNA damage leads to poly(ADP-ribosyl)ation of ABK, and subsequently inhibits ABK activity.7,48 Thus, ABK inhibition is no longer viewed as a direct mechanism of action, but rather as a consequence of other disrupted cellular processes.

Our previously unpublished data further supports the idea that ABK inhibition is not a direct mechanism of action. A direct, extra-cellular kinase activity assay of ABK in the presence of jadomycin B, F, S, or vehicle control was conducted using the ADP-Glo™ Kinase Assay (Promega) and ABK Enzyme System (Promega), as per the manufacturer’s instructions. Only 25–30% inhibitory effect was observed for 50 μM jadomycin B, F, and S in these in vitro kinase assays (Figure 4). The concentration of jadomycin B, F, and S was 10 to 50-fold higher than the IC50s in breast cancer cells (Table 2). Thus, jadomycin-mediated reductions in pH3S likely occur as a consequence of the dsDNA damage response rather than through direct ABK inhibition and new jadomycin mechanisms have been proposed to induce DNA damage, namely, topoisomerase II inhibition.

### 4.4 Topoisomerase II Inhibition

Inhibition of topoisomerasers was originally proposed as a potential mechanism based on jadomycin structural similarity to the chemotherapy DOX and the antibiotic family of fluoroquinolones, as these drugs inhibit topoisomerasers to hinder cancer and bacteria growth, respectively.12,19,49 Topoisomerasers assist in DNA replication and transcription through cleavage and realignment of positive and negative supercoiled DNA. Inhibition of topoisomerase activity can lead to DNA damage and apoptosis.7,50 There are two isoforms of topoisomerase II, topoisomerase IIa, and topoisomerase IIb, which aid in the replication of cells and neural development.51 Martinez-Farina et al. performed experiments that provided initial support for topoisomerase II as a jadomycin target.19 Using Water-Ligand Observed via Gradient Spectroscopy (WaterLOGSY), a technique used to analyze the binding affinity between enzymes and substrates, jadomycin DS was found to loosely bind human topoisomerase IIb while jadomycin LN exhibited no binding interaction.19 These findings suggested that jadomycins have the ability to bind to topoisomerase IIb, but the binding relies on jadomycin structure.19 Although this study provided the first insight into jadomycin-topoisomerase interactions, the results were somewhat limited since jadomycin DS interaction with topoisomerase IIa and other jadomycins with more established cytotoxicity profiles were not investigated.

Hall et al. conducted a series of experiments in 231-CON breast cancer cells to further explore the topoisomerase mechanism.7 The expression of TOP2A and TOP2B genes, which encode for topoisomerase IIα and topoisomerase IIβ, respectively, were significantly diminished in 231-CON cells treated with jadomycin B, F, and S versus the vehicle control.7 Jadomycin S also significantly decreased expression of the TOP1 gene encoding for topoisomerase I, but jadomycin B and F did not.7 Further investigation through western blots revealed that the topoisomerase II inhibitor MITX, and jadomycin B, F, and S, all significantly lowered the expression of topoisomerase IIα protein compared to the vehicle control in 231-CON cells.7 In 231-TXL cells, jadomycin S significantly lowered expression of topoisomerase IIα protein but MITX did not.7 Again, this verified that jadomycin S pharmacological activity was retained within the ABC- overexpressing 231-TXL cells, and was not affected by the drug efflux mechanism through which MITX was removed.7 These data suggest that jadomycins could reduce topoisomerase II function through transcription and translational mechanisms.

DNA decatenation assays demonstrated that jadomycins directly inhibited topoisomerase IIα and IIβ isoforms in a dose-dependent manner.7 The next step was to determine whether jadomycins were topoisomerase II poisons or catalytic inhibitors. Topoisomerase II poisons complex with the enzyme to directly cause dsDNA breaks, while catalytic inhibitors reduce topoisomerase activity to break
dsDNA indirectly.\textsuperscript{52} Jadomycins B, S, and F similarly inhibited topoisomerase IIα and IIβ plasmid decatenation.\textsuperscript{7} Jadomycin F was the most potent and Jadomycin B was the least potent.\textsuperscript{7} All three jadomycins were significantly less potent than DOX, suggesting they are weak and non-selective catalytic inhibitors of topoisomerase IIα and IIβ. In comparison jadomycin B and F but not S increased topoisomerase IIβ-mediated cleavage of supercoiled plasmid DNA in a manner consistent with the activity of a topoisomerase poison, albeit at low potency.\textsuperscript{7,53} Jadomycin B, F, and S did not display activity consistent with topoisomerase IIα poisoning. The nature of the selective interaction of jadomycin B and F with topoisomerase IIβ should be further explored as it could pose some concern for cardiotoxicity at higher concentrations that will need to be assessed through in vivo studies.

In summary, these findings show that jadomycins induce DNA cleavage, apoptosis, and cytotoxicity in MDR breast cancer cells through the reduction of Cu(II), production of ROS, and/or the inhibition of topoisomerase II.\textsuperscript{7,8,12} These events are hypothesized to inhibit ABK downstream, leading to cessation of mitosis and cell proliferation, and ultimately result in breast cancer cell death (Figure 5).\textsuperscript{7,9,11}

5 | LIMITATIONS AND FUTURE DIRECTIONS FOR JADOMYCIN BREAST CANCER CELL CYTOTOXICITY STUDIES

In cancer research, the study of chemotherapeutic effects in noncancerous cells and benign tumors is often excluded. This is partially due to the additional costs that such experiments would involve as well as possible redundancy, since animal models usually reveal the level of toxicity to noncancerous body cells and organ systems. It is important to note that although studies conducted thus far have verified jadomycin cytotoxicity and efficacy in MDR breast cancer cells in vitro, the effects of jadomycins in noncancerous cells remains largely unknown. Only a single study has investigated a noncancerous cell line and determined the IC\textsubscript{50} of several jadomycins in human microvascular epithelial cells (HMEC) in vitro.\textsuperscript{17} Interestingly, jadomycin Orn exhibited an appreciably higher IC\textsubscript{50} in noncancerous cells as compared to MCF7 and HCT116 cells (Table 2). This difference suggests that jadomycins can possess selectivity against different cellular targets, but it will be important for future research to establish the distribution of jadomycins to tissues and measure biomarkers of toxicity in in vivo models.

\textbf{FIGURE 5} Jadomycin proposed mechanisms of action in breast cancer cells. (A) Some jadomycins are copper-dependent and convert Cu(II) to Cu(I), leading to the production of ROS and subsequent DNA damage.\textsuperscript{8,12} (B) The DNA damage induced by jadomycins inhibits ABK and prevents mitosis and proliferation of breast cancer cells.\textsuperscript{7} (C) Jadomycins B, S, and F have also demonstrated the ability to inhibit or poison topoisomerase II, which leads to further DNA damage and apoptosis.\textsuperscript{7} Image created with BioRender.com
There is also some variability in the reported jadomycin IC_{50} values from different studies (Table 2). There could be a number of explanations for this, including methodological differences, such as the exposure time and type of assay used, or jadomycin purity and stability. Overall, however, IC_{50} values in the literature suggest that jadomycin B, F, S, and T have very similar cytotoxic profiles against cancer cells. Therefore, while different amino acids may alter jadomycin functionality, they do not always cause a significant change in potency and efficacy. As jadomycin B, F, S, and T appear to be the most cytotoxic, future development of new jadomycins should be based on these structures, with slight alterations to reduce adverse effects or improve pharmacological parameters if needed.

Moreover, while numerous studies have provided insight into jadomycin structure, chemical synthesis, and cytotoxicity, the exact cascade of events and specific cytotoxic mechanisms have yet to be determined. Research to date has focused mainly on intracellular jadomycin mechanisms and their ability to evade drug efflux transporters. Jadomycin cellular entry is an important area to explore as it will broaden the understanding of jadomycin mechanisms and allow researchers to investigate jadomycin metabolism and possible pharmacogenetic variations.

Although drug efflux is a common mechanism of MDR, the reduced uptake of transport-mediated anticancer drugs is also a major threat. Decreased uptake limits chemotherapy-induced cytotoxic DNA damage, apoptosis, and alteration of the cell cycle.54 Specifically, the role of the solute carrier transporter family should be investigated as these transporters mediate the uptake of several anticancer drugs.55 Within the family of solute carrier transporters, the organic anion transporter polypeptides OATP1B3, OATP3A1, and OATP4A1 are highly expressed in breast cancers and represents possible mechanisms for jadomycin uptake.56–58 OATP1B3 is of particular interest as it is responsible for the uptake of chemotherapies TXL, docetaxel, SN-38, methotrexate, and imatinib.56 MDR breast cancers can express different transporter phenotypes and are essential to explore as patients may have diverse pharmacokinetic responses to jadomycins.56

Ideally, we should also broaden our knowledge of less-studied jadomycins, and explore other pathways that involve jadomycins. For example, a recent study indicated that jadomycin Orn may induce more ROS production and be more efficacious than jadomycin B.59 Additional mechanisms such as the possibility of the Prx/Trx and GST/GPx antioxidant systems primarily reducing jadomycin-induced H_{2}O_{2} have been proposed, but not yet explored.8 While in vitro cell experiments are ideal for identifying initial evidence of cytotoxicity and mechanistic details, in vivo animal models are needed to observe and predict additional medical benefits, toxicities, and adverse effects. For example, as DOX is a known topoisomerase II poison and can pose serious cardiotoxic effects, jadomycins may be a desired chemotherapy alternative if they are shown to be less cardiotoxic.60,61

While the potential role of jadomycins in the treatment of MDR breast cancers is being explored, the question of how cancer cells may develop resistance to jadomycins remains. An important addition to the literature would be the development of a resistance model, to establish what changes confer a selective advantage against jadomycin treatment as this may further inform on mechanism of action.

Jadomycin pharmacogenomics is another area of interest. The MCF7, BT474, SKBR3, and MDA-MB-231 breast cancer cell lines are all derived from the breast tissues of Caucasian women, with the exception of T-47D cells for which the ethnic origins are unknown.62–65 As Gleason et al. have observed, certain breast cancer phenotypes determined through the presence and/or absence of ER and PR are more prevalent in certain ethnicities.66 Black women, regardless of age, tend to have higher rates of ER- and PR- breast cancers than White women, which reduces their therapeutic options.66 Evidence suggests that women of Chinese ethnic origins may also have a number of genetic differences in breast cancer phenotype, compared to other ethnicities, that could influence clinical care.67

Furthermore, jadomycins may exhibit anticancer activity through other factors related to ethnicity, such as the inhibition of p53 or Ki-67 protein expression, which are responsible for initiating DNA-damaged cell death and can be used to measure cell growth rate, respectively.68 Notably, the expression of Ki-67 and p53 proteins is often upregulated in triple-negative breast cancer, and the P53 gene encoding for p53 has increased expression in 30% of breast cancers.68 Significantly higher expression of both Ki-67 and p53 proteins are seen in African-American women versus Caucasian women.69 Therefore, it would be ideal to investigate the potency of jadomycins in breast cancer cell lines of various ethnic origins to compare results. This could help determine if jadomycins have an unrecognized benefit in the treatment of patients with different ethnic backgrounds.

6 CONCLUSIONS

In this review, we have presented the key findings that support jadomycins as a potential chemotherapy for MDR metastatic breast cancer. Jadomycins are repeatedly shown to avoid efflux from the overexpression of ABC-transporters and are equally cytotoxic against a variety of breast cancer cell lines regardless of hormone receptor status, which may give them an additional advantage to hormone-restricted treatment options.7 Conclusively, jadomycin B, S, and F are demonstrated to be the most potent jadomycins, and often mediate cancer cell death through the induction of apoptosis and DNA strand breaks.7,11,15 There are several jadomycin mechanisms of action that may mediate cancer cell death, including copper-dependant ROS generation, ABK inhibition, and topoisomerase II inhibition.7,8,11,12 Other mechanisms that remain to be explored include jadomycin interactions with cellular uptake transporters, jadomycin resistance, and jadomycin effects on the Prx/Trx and GST/GPx antioxidant systems and on the expression of Ki-67 and p53 proteins, which may provide additional pharmacogenomic benefits for certain patients.8,68,69 The summarized findings present jadomycins as a potential candidate for MDR breast cancer.
chemotherapy, and we strongly encourage further preclinical research in this area which may identify new and effective breast cancer treatments options.

7 NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

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DISCLOSURE

There are no conflicts of interest to report by any author.

AUTHOR CONTRIBUTIONS

Esther Bonitto developed the literature search strategy, completed the literature search, drafted the review article, and finalized the revisions. Brendan McKeown assisted with the literature search and drafting the review article, and reviewed and edited the final version. Kerry Goralski chose the topic for the review, provided supervisory support during the literature search and manuscript drafting phases, and edited and revised the drafted review article.

DATA AVAILABILITY STATEMENT

The new data that support the findings of this study (Figure 4) are available from the corresponding author Dr. Kerry Goralski upon reasonable request (kerry.goralski@dal.ca).

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