Inhibition of Mitochondrial Metabolism by (−)-Jerantinine A: Synthesis and Biological Studies in Triple-Negative Breast Cancer Cells

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COMMUNICATION

Inhibition of Mitochondrial Metabolism by (–)-Jerantinine A: Synthesis and Biological Studies in Triple-Negative Breast Cancer Cells

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A concise semi-synthesis of the *Aspidosperma* alkaloids, (–)-jerantinine A and (–)-melodinine P, and derivatives thereof, is reported. The novel compounds were shown to have potent activity against MDA-MB-231 triple-negative breast cancer cells. Furthermore, unbiased metabolomics and live cell reporter assays reveal (–)-jerantinine A alters cellular redox metabolism and induces oxidative stress that coincides with cell cycle arrest.

Nature is a wellspring of biologically active compounds primed for development into novel therapeutics. Between 1981 and 2019, approximately 23% of the first-in-class small molecules approved by the U.S. Food and Drug Administration (FDA) were natural products or derivatives thereof[1-3]. In cancer treatment, plant-derived agents like the *vinca* alkaloids (e.g., vinblastine, vincristine)[4] and taxanes (e.g., paclitaxel (Taxol))[5], remain first-line treatments, and over half of the current anticancer drugs in clinical use are derived from natural products[6]. Unfortunately, undesirable side effects and the development of drug resistance create a clinical need for novel cancer medications[7].

In 2008, Kam *et al.* isolated (–)-jerantinine A (**JA**, **1**), along with several other related *Aspidosperma* indole alkaloids, from a leaf extract of the Malayan *Tabernaemontana corymbosa*[8]. Studies revealed **JA** to exhibit *in vitro* cytotoxicity against the vincristine-resistant KB/VJ300 human cancer cell lines, with an IC_{50} value of 1.73 μM[8]. In addition, the synthetic acetate (**JA-Ac**, **2**) was also shown to exhibit comparable potency (IC_{50} = 0.83 μM) to the parent compound[9]. Subsequent studies by various laboratories have shown **JA** to have broad-spectrum activity against cell lines derived from several different human cancers (see Table S1), including aggressive triple-negative breast cancer (TNBC). Further, no cross-resistance to **JA** was observed in vincristine-resistant HCT-116 cancer cells, suggesting potential evasion of P-glycoprotein-mediated resistance mechanisms[4].

JA is an archetypical polypharmaceutical[10], operating via multiple modes of biological action. For example, **JA** induces G2/M cell cycle arrest[11] and apoptosis in cancer cell lines by disrupting microtubule polymerization (acting via the colchicine binding site)[12]. **JA** also inhibits polo-like kinase 1 (PLK1), a key regulator of mitosis[4, 11]. Leong *et al.* have linked the apoptotic effects of **JA** in MCF-7 and MDA-MB-468 breast cancer cells to disruption of spliceosome function through stabilization of the splicing factors SF3B1 and SF3B3, leading to the accumulation of unspliced pre-RNA[4].

Bradshaw and co-workers demonstrated **JA** cytotoxicity toward both estrogen receptor (ER)-positive breast cancer and TNBC cell lines (IC_{50} = 0.72–1.22 μM)[4]. In contrast, non-transformed mammary epithelial cells are relatively insensitive to **JA** (IC_{50} > 10 μM)[4], indicating a therapeutic window for selectively targeting malignant cells. The same laboratory recently reported apoferritin-encapsulated **JA-Ac** for transferrin receptor targeting, enhancing the potency relative to free **JA-Ac** by up to 14-fold against TfR1-expressing tumor cells[13].

Polypharmaceutical agents are recognized for their potential to treat diseases with complex etiology[4,5] and associated drug-resistance challenges[6]. In the context of cancer, a high degree of tumor heterogeneity predisposes patients to inferior clinical outcomes with targeted therapies. Molecules that have multiple modes of action and can impact all clonal lineages in a tumor are likely to generate the most durable response[7]. Hence, a robust synthetic supply of **JA** is warranted for further biological investigation of this promising anticancer agent.

JA comprises a densely packed pentacyclic *aspidospermidine* skeleton[14, 15], three contiguous stereogenic centers, and two quaternary carbons. One of our laboratories (Moses *et al.*) developed a sustainable semi-synthesis of (–)-**JA** from the indole alkaloid (–)-tabersonine (**TAB**, **3**) in nine

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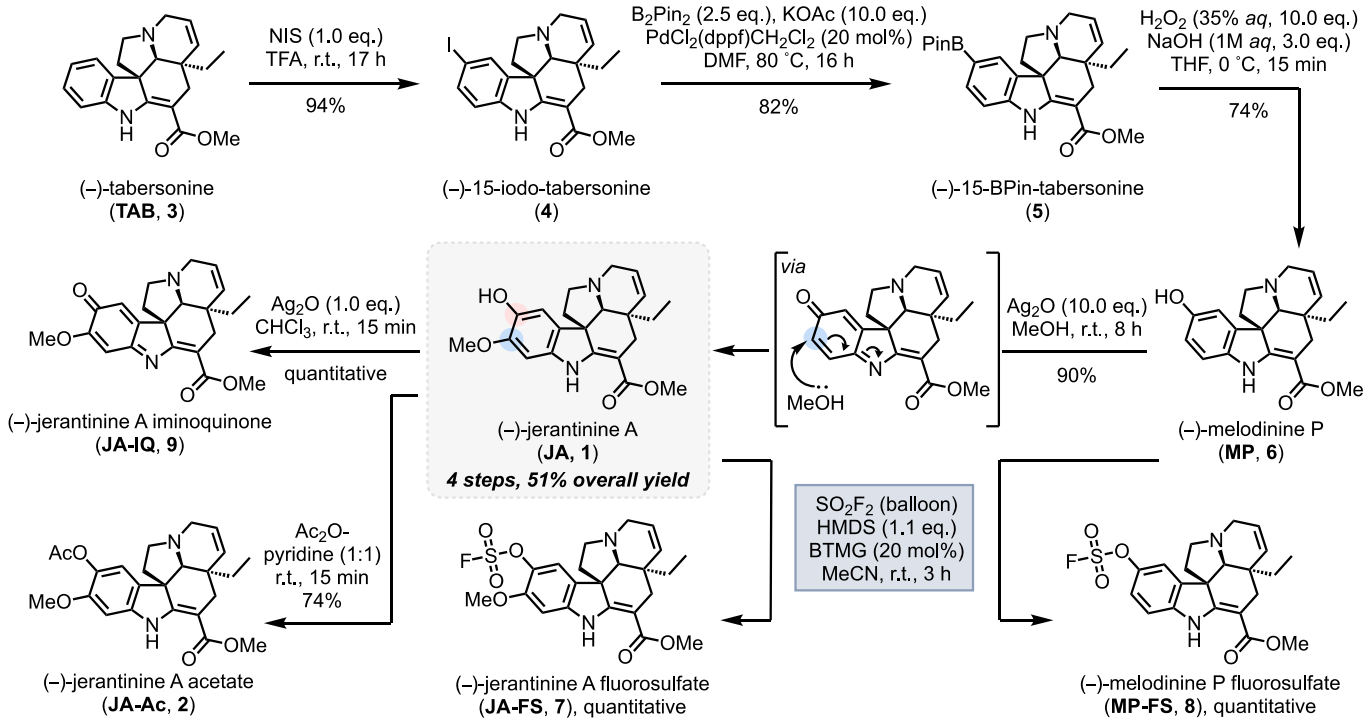

Scheme 1: Semi-syntheses of **JA** and related compounds. Refer to supplementary information for more details. Isolated yields are reported. NIS = *N*-iodosuccinimide, TFA = trifluoroacetic acid, HMDS = hexamethyldisilazane, BTMG = 2-*tert*-butyl-1,1,3,3-tetramethylguanidine.

synthetic steps and an overall yield of 29%[12]. Wang *et al.* recently developed a site-selective enzymatic oxidation of **TAB**[16], that enabled access to **JA** in up to 25% overall yield. We now report an improved four-step, protecting group-free semi-synthesis of (–)-**JA** in 51% overall yield. In addition, unbiased metabolomics studies and live-cell bioluminescent reporting systems in TNBC cells provide new insights into the biological consequences of **JA** treatment, which include the induction of mitochondrial metabolic dysfunction and oxidative stress.

Site-selective iodination of **3** to the (–)-15-iodo-tabersonine (**4**) occurred in 94% yield (Scheme 1), followed by Miyaura borylation to (–)-15-BPin-tabersonine (**5**) in 82% isolated yield. The oxidative hydrolysis of **5** with hydrogen peroxide and sodium hydroxide in THF afforded the intermediate **6** in 74% yield, itself a secondary metabolite from *Melodinus suaveolens*[17], a plant used in traditional Chinese medicine, named (–)-melodinine P (**MP**, **6**). Finally, selective installation of the C-16 (blue carbon) methoxy group was achieved by stirring **6** with silver(I) oxide in methanol at room temperature, delivering the target (–)-**JA** in 51% overall yield from **TAB**.

Upon exposure to the atmosphere, **JA** slowly oxidizes to the unstable iminoquinone (**JA-IQ**, **9**), which undergoes decomposition. The corresponding acetate (**JA-Ac**, **2**)[8] is however bench stable. **JA-IQ** can be synthesized by oxidizing **JA** with 1.0 equivalent of silver oxide in chloroform[18]. Wu and co-workers have shown that the fluorosulfate derivatives of some anticancer agents can have significantly improved stability and bioactivity[19]. Hence, we prepared the fluorosulfates **JA-FS** (**7**) and **MP-FS** (**8**) from **JA** and **MP**, under accelerated SuFEx click chemistry conditions with SO_2F_2[20].

With significant quantities of materials in hand, we next performed a series of biological studies using an aggressive TNBC cell line as a model. TNBC is a highly aggressive and invasive subtype of breast cancer, characterized by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2)[21]. TNBC is a rapidly progressive cancer, with early-onset metastatic disease, visceral metastases, and short response duration to available therapies, resulting in inferior survival outcomes[22]. Since TNBC cells lack ER & PR receptors and express limited HER2, the cancer is non-responsive to hormone therapy and targeted HER2 drugs, leading to substantially fewer treatment options for patients.

We first quantified the antiproliferative activity of **JA**, **MP**, and related compounds **JA-Ac**, **JA-FS**, **MP-FS**, **JA-IQ**, and **TAB** against the MDA-MB-231 TNBC cell line (ATCC) (Figure 1). All compounds were cytotoxic, with **JA**, **JA-IQ**, and **MP** eliciting the most potent antiproliferative response (IC_{50} = 1.92, 1.96, and 1.06 µM, respectively). The fluorosulfates **JA-FS** and **MP-FS** were less potent than their parent compounds, with 2–6-fold higher IC_{50} values noted. This may suggest that the *in situ* oxidation of **JA** and **MP** to iminoquinone form contributes to the observed biological activity[23].

Aligning with other reports, **JA-Ac** had similar activity (IC_{50} = 2.60 µM) to **JA** against MDA-MB-231 cells[13]**.** Notably, when non-transformed MCF-10A human mammary epithelial cells (ATCC) were treated with **JA-Ac**, a ~9-fold increase in the IC_{50} value of 18.77 µM was observed (Figure S1A), while, in our hands, **JA** maintained its potency (IC_{50} = 0.96 µM, Figure S1B)[24, 25]. While jerantinine acetates have themselves been characterized as cytotoxic colchicine site binders of β-tubulin[12], we speculate that the increased activity of **JA-**

Ac in MDA-MB-231 cells compared to MCF-10A cells is the result of a prodrug effect. Cancer cells are known to express elevated levels of esterases[23], which could increase the rate of ester hydrolysis and release of the more cytotoxic **JA**, and ultimately **JA-IQ**, in TNBC cells relative to MCF-10A cells.

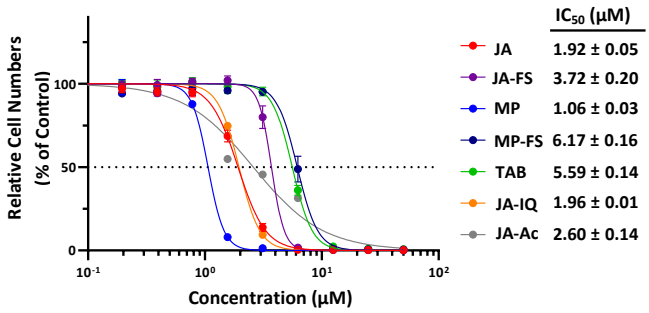

Figure 1. Growth inhibitory effect of **JA** and its derivatives against the MDA-MB-231 breast cancer cell line. Cell numbers were quantified by CyQUANT assay after 5 days of treatment at indicated concentrations (error bars indicate SEM; n=4). IC_{50} values were calculated using GraphPad Prism.

To explore the biological activities of the semi-synthetic molecules, we applied live-cell imaging assays using bioluminescent oxidative stress reporters. MDA-MB-231 cells were transfected with a single lentiviral vector (OxiLuc) constructed to produce both red and green colored bioluminescence[26, 27], and compounds were dosed with increasing concentrations (from 0 to 40 μM) for a period of 24 hours. The treatment of **JA** and **MP** resulted in a substantial increase in the green bioluminescence, indicative of the accumulation of reactive oxygen species, whereas the compounds **3**, **7**–**9** failed to elicit a robust response (Figure 2).

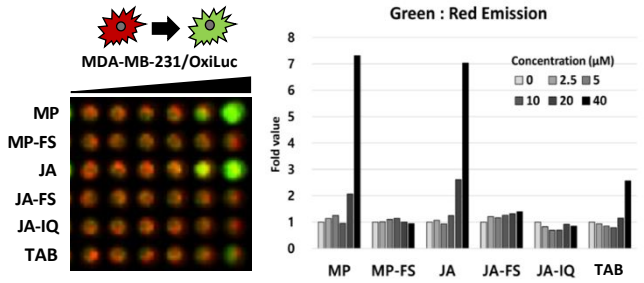

Figure 2. *In vitro* imaging assays detecting oxidative stress. Increase in green color indicates ROS generation.

We next performed liquid chromatography-mass spectrometry (LC-MS)-based metabolomics to evaluate the impact of **JA** and **JA-FS** on cellular metabolism of MDA-MB-231 cells. **JA-IQ** was omitted from this assay due to its instability. We found that treatment of MDA-MB-231 TNBC cells with 5 μM **JA** for 16 h induced profound changes in intracellular metabolite abundances. In contrast, the less potent **JA-FS** derivative at the same concentration, caused relatively modest perturbations, and clustered close to the vehicle-treated control samples (Figure 3A, 3B and S2). An enrichment analysis identified several redox and amino acid metabolism pathways among those most impacted by **JA** treatment (Figure S3 and Table S2). Notably, treatment with **JA** (but not **JA-FS**) caused a marked decrease in intracellular levels of aspartate, an amino acid that is a limiting metabolite for cellular proliferation[28]. Correspondingly, levels of aspartate-derived metabolites, including argininosuccinate, *N*-acetylaspartate, and carbamoyl aspartate (a key intermediate of *de novo* pyrimidine biosynthesis) also decreased by up to 90% (Figure 3C). Consistent with inhibition of pyrimidine biosynthesis by **JA**, levels of cytidine, an intermediate of the pyrimidine salvage pathway was elevated approximately 10-fold (Figure 3C). Supporting the notion that **JA** impacts cellular metabolism, a previous RNAi screen identified major changes in the essentiality of genes related to arginine and proline metabolism, processes that primarily occur in mitochondria[4]. Collectively, our results implicate **JA** as an inhibitor of cellular nucleotide metabolism and support future investigation of the molecular targets that elicit this effect.

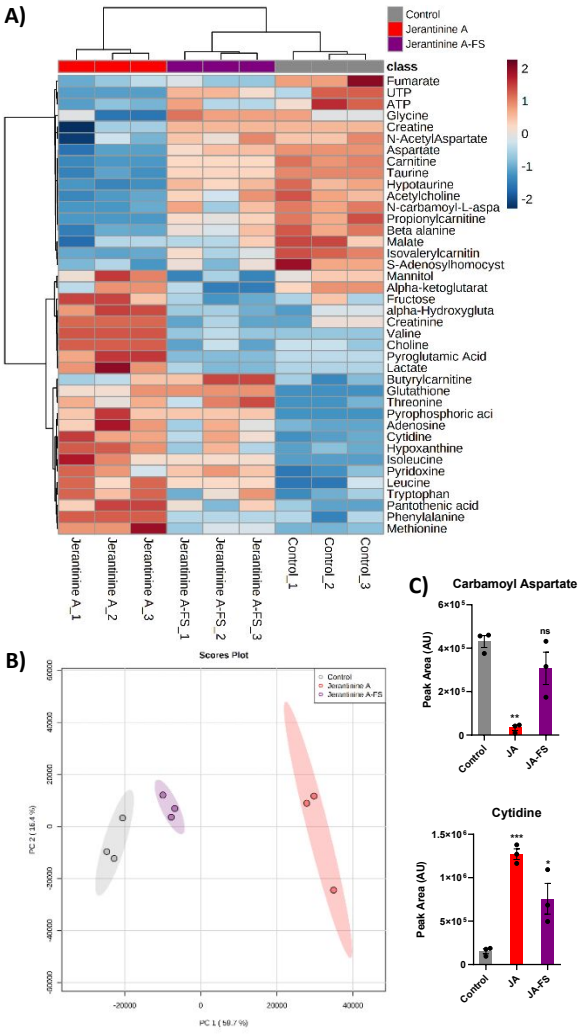

Figure 3. A) Heatmap analysis of top 40 metabolites differentially abundant after ANOVA test in comparison between 5 μM (–)-jerantinine A (**JA**, **1**), 5 μM (–)-jerantinine A fluorosulfate (**JA-FS**, **7**), and vehicle control. The colors indicate relative fold change of each metabolite between groups (orange: higher; blue: lower abundances). **B)** Principal Component Analysis (PCA) score plot of MDA-MB-231 treated with 5 μM **JA**, 5 μM **JA-FS**, or vehicle control. Colored regions display 95% confidence. **C)** Bar plots of key metabolites with peak area on y-axis and mean±SEM. Asterisks indicate significant differences between control and experimental groups, * <0.05, ** <0.01, *** < 0.001, ns: not significant.

In summary, a concise semi-synthesis of (–)-jerantinine A and (–)-melodinine P is reported. The anticancer activity of these natural products along with a selection of synthetic derivatives, including **JA-Ac**, **JA-FS**, **MP-FS**, **JA-IQ**, and **TAB**, was evaluated against MDA-MB-231 TNBC cells. Both **JA** and **MP** demonstrate potent antiproliferative activity (IC_{50} = 1–2 μM) against TNBC cells, whereas the fluorosulfate derivatives (**7** and **8**) were less active (IC_{50} = 3–6 μM). The bench-stable **JA-Ac** showed enhanced selectivity for TNBC cells over non-transformed mammary cells. Furthermore, metabolomics analysis revealed that **JA** is a potent inhibitor of nucleotide metabolism. Collectively, this study, along with the work of others, supports the development of **JA** and **JA**-derivatives as promising polypharmaceuticals for the treatment of cancers that currently lack effective therapies.

Acknowledgements

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Conflicts of interest

There are no conflicts to declare.

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