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Fusarisetins: Structure-function studies on a novel class of cell migration inhibitors

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Abstract

Herein, we report the effects of fusarisetin A on cell morphology focusing in particular on actin and microtubules dynamics. We also report the synthesis and structure-function studies of a designed library of synthetic fusarisetins in cell-based assays.

While there have been tremendous advances in cancer research and treatment, the mortality caused by this disease represents still an enormous challenge.¹ In fact, according to the American Cancer Society, more than 1.5 million new cancer cases are diagnosed every year and more than 0.5 million Americans will die annually from this disease.² About 90% of these deaths are attributed to cancer metastasis, which is the ability of tumor cells to migrate from their tissue of origin and colonize elsewhere in the body.³ When cancer is detected at an early stage, before it has spread, it can often be treated successfully either by local therapy (surgery, radiation) or by systemic therapy (chemotherapy, targeted therapy, hormonal therapy).⁴ However, when cancer is detected after it has metastasized, such treatments are much less successful. Furthermore, for many patients for whom there is no evidence of metastasis at the time of their initial diagnosis, metastatic tumors could be detected at a later time.⁵ Thus, there is a clear need to develop novel and effective treatment strategies that target tumor progression by interfering with the metastatic process.

In principle, it is possible to halt (or retard) cancer metastasis at different stages with the help of cell migration inhibitors.⁶ A significant benefit of identifying such inhibitors stems from the observation that they do not need to be cytotoxic and thus they do not produce side effects associated with the use of current chemotherapeutic agents. This approach has led to drug discovery strategies that affect cancer metastasis by targeting critical regulators of extracellular matrix, cell motility and adhesion.^{7,8} These efforts demonstrate the great therapeutic potential of targeting cancer metastasis and the urgent need to identify potent inhibitors of this process. In the recent literature only few examples exist of natural product

anti-migration agents that have been synthetically pursued leading to their evaluation in different biological models.⁹

Screening efforts to identify novel cell migration inhibitors led to the isolation of fusarisetins A (**1**) and B (**2**) (Figure 1). Isolated from a *Fusarium* species, these fungal metabolites were found to inhibit acinar morphogenesis in MDA-MB-231 cells, a highly metastatic breast cancer cell line.¹⁰ Their peculiar biological activity, in combination with their novel pentacyclic motif, has attracted the attention of the synthetic community. To-date, several total syntheses of these natural products have been described in the literature.^{11–14} We have recently reported a scalable synthesis of fusarisetins,¹⁵ which yielded significant amounts of synthetic material in enantiopure form (greater than 95% ee) paving the way for a methodical structure-function study presented herein.

At the onset of this investigation, we evaluated the effects of **1** on the cell morphology and function. In general, motility inhibitors are known to interfere with microtubules,¹⁶ actin¹⁷ and/or cell adhesion processes.¹⁸ For instance cytochalasin D (**3**), a natural product that binds to actin filaments and induces actin depolymerization, is a well-known cell motility inhibitor.¹⁹ Intrigued by the observation that **3** is structurally and biogenetically related to **1**,²⁰ we sought to compare their effects *in vitro*. Incubation of cells with **3** induced the expected actin depolymerization as shown by intense staining of monomeric actin and lack of actin fibers (Figure 2, column A). This effect was reversible, since removal of **3**, by washing the cells with PBS and re-incubation with vehicle control, led to recovery of the actin network (Figure 2, column B). Removal of **3** followed by re-incubation with growth media containing **1**, led to reconstitution of actin filaments (Figure 2, column C), indicating that fusarisetin A does not affect actin polymerization. Similar observations were made upon sole treatment of cells with **1** (Figure 2, column D). Moreover, in all cases we observed healthy microtubules morphology (Figure 2). Based on these findings we can conclude that fusarisetin A does not affect actin nor microtubules dynamics. These results, parallel previous findings¹⁰ and further support the notion that fusarisetin A acts via a novel mechanism of action that is distinctly different from those of known anti-migration agents.

At present, the key structural features of fusarisetins that account for their anti-motility properties are unknown. To address this issue, we sought to construct a library of analogs in which key reactive sites of **1** were systematically evaluated for their bioactivity. Interestingly, incorporation of various amino acids has been shown to occur in biosynthetically related natural products that derive from the same gene cluster of *Fusarium* species.²⁰ We began this study by exploring the biological significance of the *N*-methyl serine moiety in fusarisetin A by varying the amino acid motif (C3 modification) (Scheme 1). Our synthetic strategy towards **1** allows coupling of *N*-methyl serine with β -keto acid **4**, and conversion of the resulting amide to fusarisetin A via a key oxidative radical cyclization (ORC) reaction cascade. Using this strategy, β -keto acid **4** was coupled with the *N*-methyl amino methyl esters of phenyl alanine (**5a**), alanine (**5b**), isoleucine (**5c**) and glycine (**5d**) to produce compounds **6a–6d** respectively. Treatment of these adducts with NaOMe led to tetramic acid analogs **7a** to **7d** (86–94% over 2 steps). It is worth noting that during this Dieckmann condensation we observed only minimal epimerization at the C3 center. On the other hand, significant racemization at the C3 center was observed when a serine analog was

cyclized under the same conditions en route to the synthesis of **1**.¹⁵ This difference is attributed to the methyl hydroxy group of serine that inductively increases the acidity of the C3 proton.²¹ Exposure to cerium ammonium nitrate (CAN) in acetic acid under oxygen atmosphere followed by reduction of the resulting endoperoxides,²² produced fusarisetin analogs **8a–8d** (18–25% overall yield).

The AB decalin ring system of fusarisetins is also present in a wide array of natural products.²³ It was hypothesized that the biological properties of **1** arise from the unique architectural motif of its CDE ring system. We also speculated that the stereochemistry at the C5 center is not critical to the fusarisetin bioactivity since both fusarisetin A (**1**) and B (**2**) are equipotent. With this in mind, we developed a synthesis of truncated analog **12** containing a C5 dimethylated center that, in turn, simplifies the stereoisomeric ratio obtained during the ORC reaction (Scheme 2). Compound **12** was synthesized via the following sequence: (a) kinetic alkylation of ethylacetoacetate (**9**) with prenyl bromide to form **10**; (b) saponification of the ethyl ester followed by coupling of the resulting carboxylic acid with **5a** to produce **11** (60% yield over 3 steps); (c) Dieckmann condensation (NaOMe/MeOH); and (d) ORC reaction (CAN, O₂) followed by endoperoxide reduction (CuCl) to form **12** (34% yield over 2 steps).

We then explored the structure/function relationship of the decalin system of **1** by functionalizing the C₈–C₉ alkene (Scheme 3). OsO₄-catalyzed dihydroxylation produced diol **13** (58% yield). The stereoselectivity of this reaction has been unambiguously confirmed by X-ray structure analysis.¹⁰ Similarly, treatment of **1** with *m*CPBA selectively afforded epoxide **14** (68% yield). Reduction of the C₈–C₉ alkene proceeded under Pd-catalyzed hydrogenation conditions to afford saturated analog **15** (95% yield). On the other hand, acetylation of the C18 hydroxyl group produced acetate **16** (66% yield).

Initial cell-based evaluation of all fusarisetins (synthetic material,¹⁵ 1–100 μM) was performed using a well-described scratch wound assay.²⁴ MDA-MB-231 cells were grown as a confluent monolayer, scratched and treated with analogs over a 24 hour period. Compounds **8a–8d** proved to be inactive up to 100 μM concentrations suggesting that the hydroxymethyl group of serine plays a significant role in the biological activity of **1**. Truncated fusarisetin **12**, representing the CDE motif, was also inactive in the scratch wound assay even at high μM concentrations (Table 1). Among the synthetic fusarisetin derivatives (compounds **13–16**) neither epoxide **14** nor reduced analog **15** showed any activity *in vitro*. However, dihydroxylated derivative **13** and acetate **16** demonstrated inhibition of wound healing as shown in Figure 3. These compounds were then subjected to a Boyden chamber Transwell assay to quantitatively determine their anti-motility activity (Figure 4). The IC₅₀ values of fusarisetin analogs are shown in Table 1.

Figure 5 summarizes the observed structure-function relationship of fusarisetins. Comparison of the bioactivity data indicates that: (a) both the AB decalin motif and the serine amino acid are critical to the biological profile of fusarisetins; (b) the C5 stereochemistry is insignificant to the activity; and (c) although the B ring alkene is significant to bioactivity, its dihydroxylation can lead to analogs that maintain the

bioactivity albeit at a higher concentration. Similarly, acetylation of the C18 oxygen produces a compound that inhibits cell migration at high μ Molar concentration.

Conclusions

In summary, we present our studies toward deciphering the pharmacophoric motif of fusarisetins, a new family of potent cell migration inhibitors. Fusarisetin A, the archetype of this family, inhibits cell motility without directly targeting actin or microtubule networks. Empowered by our robust synthetic strategy,^{12,15} we have produced several analogs that were used to interrogate the biological significance of the fusarisetin framework. We found that structural modifications of this rigid scaffold, such as deletion of the AB ring system or replacement of the E-ring serine with other amino acids, result in acute loss of potency. However, subtle changes at the periphery of the fusarisetin motif, such as shuffling of the stereochemistry at the C5 center, result in retention of activity. Moreover, acetylation of the C18 hydroxyl group or dihydroxylation of the C8-C9 alkene produces compounds that maintain biological function albeit at higher concentration, suggesting that these functionalities could be used as tethering sites for further functionalization. Our observations strongly support the notion that fusarisetins operate via an unexplored mechanism of action associated with cell motility. Importantly, these findings attest to the uncharted and highly promising potential of fusarisetins as novel leads for the development of cancer metastasis inhibitors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Selected natural products that inhibit cell migration

Figure 2. Effect of cytochalasin D (3**) and fusarisetin A (**1**) on actin and microtubule dynamics** MDA-MB-231 cells stained for actin (phalloidin, green), microtubules (β -tubulin, red) and nuclei (DAPI, blue). Cells treated with **3** (4 h, 10 μ g/mL) (column A); were washed with PBS to remove **3** and then incubated with: vehicle control (column B); or **1** (12 h, 10 μ g/mL) (column C). Cells treated solely with **1** (12 h, 10 μ g/mL) (column D).

Figure 3.

Evaluation of synthetic fusarisetins in a scratch wound assay. MDA-MB-231 cells, grown as a confluent monolayer, were scratched to generate a cell-free zone and photographed at time 0 (A). Cells were incubated for 24 hrs with DMSO as control (B) and in the presence of compounds **13** (100 μ M) (C) and **16** (100 μ M) (D).

Figure 4.

Dose-dependent inhibition of cell migration by **13** and **16** in a Transwell assay. Cells were plated in a Boyden chamber with **13**, **16** or vehicle control (DMSO) for 24 h at the indicated concentrations. Amount of migrated cells is presented relative to the corresponding DMSO control.

Figure 5.
Structure-function relationship map of fusarisetins.

Scheme 1.

Synthesis of C3 analogs via a peptide coupling with diverse *N*-methyl amino ester fragments followed by ORC/reduction sequence

Scheme 2.
Synthesis of CDE core fusarisetin analog **12**

Scheme 3.
Synthesis of derivatives of fusarisetin A

Table 1IC₅₀ values of fusarisetin analogs

Compound	IC ₅₀ (μM)
1	7.7 ¹⁵
2	7.7 ^b
8a	> 100
8b	> 100
8c	> 100
8d	> 100
12	> 100 ^c
13	74.5 ± 3.2
14	> 100
15	> 100
16	85.3 ± 3.8

^aCompounds with no significant activity in the scratch wound assay (up to 100 μM) were not submitted to the Transwell assay. IC₅₀ values of compounds **13** and **16** were determined by the Transwell assay.

^bValue obtained from references 10 and 15.

^cCompound **12** was tested as the racemate.