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EDGE ARTICLE

Fusarisetin A: scalable total synthesis and related studies†

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Fusarisetin A (**1**) is a recently isolated natural product that displays an unprecedented chemical motif and remarkable bioactivities as a potent cancer migration inhibitor. We describe here our studies leading to an efficient and scalable total synthesis of **1**. Essential to the strategy was the development of a new route for the formation of a *trans*-decalin moiety of this compound and the application of an oxidative radical cyclization (ORC) reaction that produces fusarisetin A (**1**) from equisetin (**2**) *via* a bio-inspired process. TEMPO-induced and metal/O_2-promoted ORC reactions were evaluated. Biological screening *in vitro* confirms the reported potency of (+)-**1**. Importantly, *ex vivo* studies show that this compound is able to inhibit different types of cell migration. Moreover, the C_5 epimer of (+)-**1** was also identified as a potent cancer migration inhibitor, while (−)-**1** and **2** were found to be significantly less potent. The optimized synthesis is applicable on gram scale and provides a solid platform for analogue synthesis and methodical biological study.

Introduction

Despite the tremendous advances, cancer still represents an enormous medical challenge since, only in America, it is responsible for more than half a millions of deaths per year.[1] 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.[2] When cancer is detected at a premetastatic stage, it can often be treated successfully either by local therapy (surgery, radiation) or by systemic therapy (chemotherapy, targeted therapy, hormonal therapy).[3] However, when it is detected after it has metastasized, such treatments are much less successful. Along these lines, metastasis is considered as the "last frontier" in cancer management for which, to-date, there is no effective treatment.[4,5]

In principle, it is possible to halt (or retard) cancer metastasis with the help of small molecules that inhibit cell migration.[6] Recent advances in high-throughput screening and high content imaging techniques permit the identification of new cancer metastasis inhibitors from libraries of natural products or small molecules.[7,8] On the other hand, the availability of complex natural products *via* improved isolation techniques and streamlined synthetic strategies (or genetic engineering) allows evaluation of their effect in cell migration assays. Sceptrin[9] and migrastatin[10] represent a few recent examples of such efforts. Importantly, the development of scalable syntheses of these compounds[11,12] have resulted in the development of new chemical tools for the study of proteins involved in cancer metastasis and the discovery of potent cell migration inhibitors for further preclinical studies.[11b,12c,d]

Recent efforts to identify potent inhibitors of cancer metastasis have led to the isolation of fusarisetin A (**1**) from a *Fusarium* species (Fig. 1).[13] This compound was found to inhibit cancer metastasis in MDA-MB-231 cells, a particularly aggressive breast cancer cell line. Specifically, **1** was found to inhibit acinar morphogenesis (IC_{50} *ca.* 77 μM), cell migration (IC_{50} *ca.* 7.7 μM) and cell invasion (IC_{50} *ca.* 26 μM) in these cell lines without any significant cytotoxicity in concentrations up to 77 μM. Interestingly, the proteomic profiling of **1** was found to be significantly different to those of other reference compounds. Moreover, **1** did not inhibit the phosphorylation of ERK1/2, AKT, c-Jun and p38 kinases in response to EGF treatment, as is commonly observed with compounds that inhibit cancer metastasis by altering protein kinases.[6] These findings suggest that the molecular target of fusarisetin A is different from those of known compounds and thus, its identification could produce new fundamental knowledge in the signal transduction pathways related to cancer metastasis. From a chemistry standpoint, fusarisetin A possesses an unprecedented pentacyclic ring system of which the CDE rings contain various polar functionalities (ketone, lactam, hemiketal and primary alcohol). The combination of impressive chemical structure and potent bioactivity is evidenced by the three publications describing various syntheses of **1** in about one year after its structure became known in the literature.[14–16]

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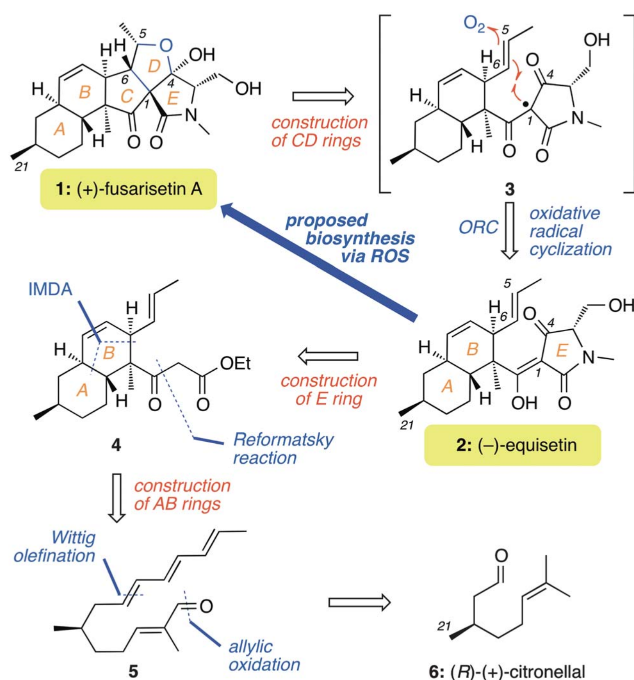

Fig. 1 Retrosynthetic analysis of fusarisetin A (**1**) *via* equisetin (**2**) featuring a bioinspired oxidative radical cyclization (ORC) reaction (ROS: reactive oxygen species).

Intrigued by this molecule, we devised a project focused on the development of a scalable chemical synthesis that may allow the study of its chemical biology. Herein we report a detailed account on the synthesis of fusarisetin A. The developed strategy is short, efficient and stereoselective and is highlighted by the use of a key oxidative radical cyclization (ORC) reaction that allows conversion of equisetin (**2**) to fusarisetin A (**1**) following a likely biosynthetic pathway. Moreover, our biological studies confirm the reported bioactivity of **1** *in vitro* and indicate that this activity is maintained in whole tissue using *ex vivo* assays.

Results and discussion

Retrosynthetic analysis and strategic bond disconnections

Close inspection of the fusarisetin framework reveals the fusion of a *trans*-decalin unit (AB ring system) with a tetramic acid moiety (E ring). These rings can also be found in the structure of equisetin (**2**),[17] another secondary metabolite produced by a *Fusarium* species, suggesting that both molecules may arise from a common biosynthetic pathway (Fig. 1).[18] Along these lines, we hypothesized that **1** derives biogenetically from oxidation of **2** upon exposure to reactive oxygen species (ROS).[19] This biosynthetic scenario could account for the formation of stabilized radical **3** that, upon cyclization at the pendant alkene followed by trapping by ROS and hemiketalization, would produce **1**. Translating this proposal to a synthetic plan, we envisioned that equisetin could undergo a 5-*exo*-*trig* oxidative radical cyclization (ORC)[20] to form the C_1–C_6 bond. The resulting C_5 radical could then be trapped by oxygen or a related ROS. Further bond disconnection of the equisetin motif suggested that **2** could be produced from β-ketoester **4**, the *trans*-decalin motif of which (AB ring system) could be made *via* an intramolecular Diels–Alder reaction (IMDA).[21] This scenario led us to consider polyene **5** as the potential precursor of the IMDA. In turn, **5** could be synthesized from commercially available citronellal (**6**) whose motif contains the C_{21} methyl group with the desired stereochemistry.[22]

Synthesis of the *trans*-decalin moiety and evaluation of the TEMPO-induced ORC

The retrosynthetic plan shown in Fig. 1 calls for departure of the synthesis from (*R*)-(+)-citronellal (**6**). Nonetheless, guided by the original assignment of fusarisetin A,[13] we started our synthesis with commercially available (*S*)-(−)-citronellal (***ent*-6**) (Scheme 1).[23] Inspiration for our studies came from previously reported syntheses of equisetin by the Danishefsky, Dixon and Shishido groups.[24] With an eye toward step-economy,[25] we sought to develop an alternative synthesis of this compound. To this end, ***ent*-7** was synthesized from ***ent*-6** *via* cross-metathesis with methacrolein using a Ru-carbene catalyst (Grubbs 2nd generation, 5 mol%)[26] (75% yield). Alternatively, allylic oxidation of ***ent*-6** with SeO_2/IBX can also form ***ent*-7** in 65% yield. Although both reactions are scalable, in our subsequent studies we used the SeO_2/IBX method due to its lower cost. The two chemically differentiable carbonyl groups of this compound provide the possibility to install the polyene motif in a regioselective manner. Initial olefination studies of ***ent*-7** under HWE or Julia conditions proved to be unsatisfactory.[24b,c,27] However, slow addition of the Wittig ylide, generated upon deprotonation of phosphonium salt **8**,[28] to ***ent*-7** afforded polyene ***ent*-5** in 62% overall yield as a mixture of *E*–*Z* isomers ($E : Z = ca.\ 3 : 2$). Photo-induced isomerisation of this mixture with catalytic amount of iodine[29] produced exclusively the *trans* polyene. Without purification, this compound was subjected to a Et_2AlCl-promoted IMDA reaction, that stereoselectively produced the desired *trans*-decalin aldehyde ***ent*-9** (dr > 10 : 1, 82% yield). It is noted that the rapid construction of this *trans*-decalin motif could grant access to other biologically interesting natural products.[30] Treatment of ***ent*-9** with ethyl bromoacetate under Reformatsky conditions followed by IBX oxidation yielded β-ketoester ***ent*-4** in 91% combined yield. Aminolysis of this compound with serine derivative ***ent*-10**, followed by deprotection of the TBS group, produced ***ent*-11** (43% yield overall). Dieckmann condensation of ***ent*-11** produced a mixture of (+)-equisetin (***ent*-2**) together with its C_3-epimer (***ent*-C_3-*epi*-2**) (100% yield, dr = 1 : 1).[22] It should be noted that the tendency of equisetin to epimerize at the C_3 center under basic conditions has been previously reported[31] and has been observed consistently in our studies. Low-temperature ^{1}H NMR experiments have also confirmed that equisetin exists exclusively in the enol form and not as a tautomeric mixture as recently reported.[16] The structures of these compounds were also confirmed by comparison with the known data.[16,17,24,31]

With ***ent*-2** in hand, we sought to explore ORC processes for the formation of the C ring of fusarisetin. It is worth noting that although radical reactions have often been used in natural products synthesis for the construction of C–C bonds,[32] their application to the formation of C–O bonds remains limited.[33] A report by Jahn *et al* on the construction of five-membered rings, using 1,3-dicarbonyl groups and alkenes under TEMPO

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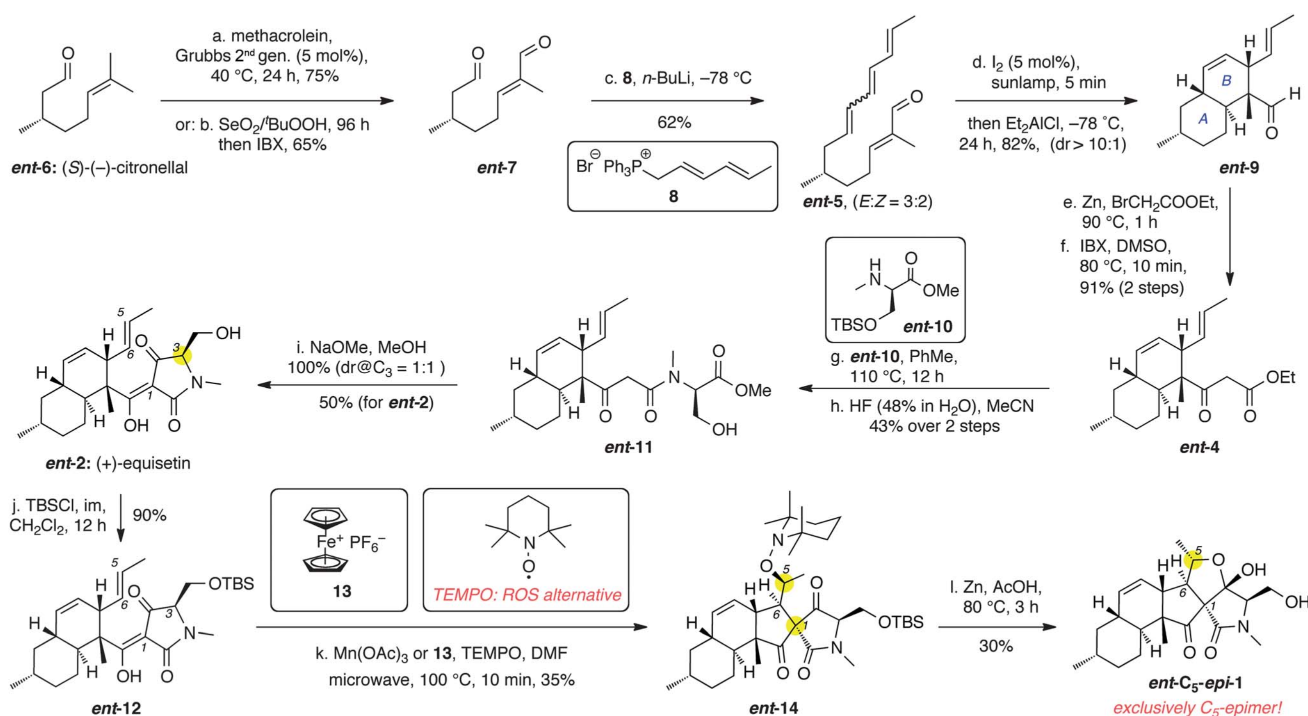

Scheme 1 Synthesis of the *trans*-decalin moiety and study of the TEMPO-induced ORC. *Reagents and conditions*: (a) methacrolein (2.0 equiv.), Grubbs 2nd gen. catalyst (5 mol%), CH_2Cl_2, 50 °C, 24 h, 75% (90% based on recovered starting material); (b) SeO_2 (3 mol%), ^tBuOOH (4.0 equiv.), salicylic acid (0.1 equiv.), CH_2Cl_2, 96 h, then IBX (1.4 equiv.), DMSO, 1.5 h, 65%; (c) **8** (1.0 equiv.), *n*-BuLi (1.0 equiv.), THF, −60 °C, 1 h, then −78 °C, ***ent*-7**, (see ESI†), 62%; (d) I_2 (5 mol%), sunlamp (visible light), CH_2Cl_2, 5 min, then −78 °C, Et_2AlCl (1.0 equiv.), 24 h, 82%; (e) activated zinc dust (3.0 equiv.), ethyl bromoacetate (1.2 equiv.), PhH, 45 min, 90 °C; (f) IBX (2.0 equiv.), DMSO, 80 °C, 10 min, 91% for two steps; (g) **10** (3.0 equiv.), PhMe, reflux, 12 h; (h) HF (48% in H_2O, 10 equiv.), MeCN, 15 min, 43% over two steps; (i) NaOMe (5.0 equiv.), MeOH, 10 min, 100 % combined yield (dr = *ca.* 1 : 1); (j) TBSCl (1.5 equiv.), imidazole (2.0 equiv.), CH_2Cl_2, 12 h, 90%; (k) **13** or $Mn(OAc)_3$ (2.0 equiv.), TEMPO (3.0 equiv.), DMF, microwave, 100 °C, 35%; (l) activated zinc dust (20 equiv.), AcOH–THF–H_2O (3 : 1 : 1), 80 °C, 3 h, 30%.

conditions, provided a possible way for the desired transformation.[34] However, our initial studies with ***ent*-2** gave unsatisfactory results, presumably due to the sensitivity of its C_3 hydroxymethyl group. To overcome this issue, we protected equisetin as its TBS ether ***ent*-12** (90% yield). Gratifyingly this compound underwent the desired ORC, using ferrocenium hexafluorophosphate (**13**) or Mn(III) acetate as the oxidants,[35] to afford cyclized TEMPO-product ***ent*-14**, albeit in moderate yield (35%). Mechanistically, this reaction proceeds *via* a heat-promoted homolytic cleavage of the TEMPO–C_1 bond.[36] The resulting stabilized radical at C_1 reacts with the pendant C_5–C_6 alkene to generate the C_5-radical that can subsequently be trapped by the available TEMPO.[37] Reduction of the alkoxylamine bond of ***ent*-14** under Zn/AcOH conditions[38] liberated the C_5-alcohol that underwent the desired hemiketalization, along with concomitant deprotection of the TBS group, to form a compound that was spectroscopically identified as the C_5-epimer of (−)-fusarisetin A (***ent*-C$_5$-*epi*-1**).[14]

The results of this study allowed us to draw several conclusions related to the TEMPO-mediated ORC reaction. As predicted, the TEMPO can indeed act as an ROS synthetic alternative and could form the C ring of ***ent*-14** albeit in low yield. Gratifyingly, the stereochemistry of the C_1–C_6 bond was efficiently controlled by the structure of the decalin ring. Unfortunately, the stereochemistry of the C_5 center was not the desired one. Moreover, we encountered difficulties applying this reaction to a non-protected equisetin (***ent*-2**). These considerations prompted us to apply the TEMPO-mediated ORC on a less functionalized substrate.

Total synthesis of (−)-fusarisetin A (*ent*-1)

β-Keto ester ***ent*-4** appeared to be an attractive substrate for the TEMPO-mediated ORC, since it is less functionalized than equisetin and also contains an easily oxidizable C_1 center. With this in mind, ***ent*-4** was treated with LiHMDS and the resulting C_1 enolate was *in situ* oxidized with **13** to afford, after quenching of the C_1 radical with TEMPO, compound ***ent*-15**. As expected, under these conditions (5 min, 0 °C) the ORC did not occur and ***ent*-15** was isolated and fully characterized as a mixture of C_1-isomers (*ca.* 2.5 : 1) in 99% yield. Heating this isomeric mixture at 90 °C over a period of 36 h gave rise to the tricyclic motif of ***ent*-16** *via* the desired 5-*exo*-*trig* cyclization. Similarly with the above study, the formation of the C_1–C_6 bond proceeded with excellent stereocontrol, presumably due to the stereochemical bias of the decalin motif. Interestingly however, in this case we obtained a mixture of stereoisomers at C_5 (*ca.* 1 : 1). It is worth mentioning that attempts to decrease the reaction time by raising the temperature proved to be problematic since they led to significant amounts of decarboxylated product ***ent*-17**. To further enhance the overall efficiency, we also examined the one-pot ORC and aminolysis sequence in presence of serine derivative ***ent*-18** (Scheme 2). To our delight, this one-pot reaction gave rise

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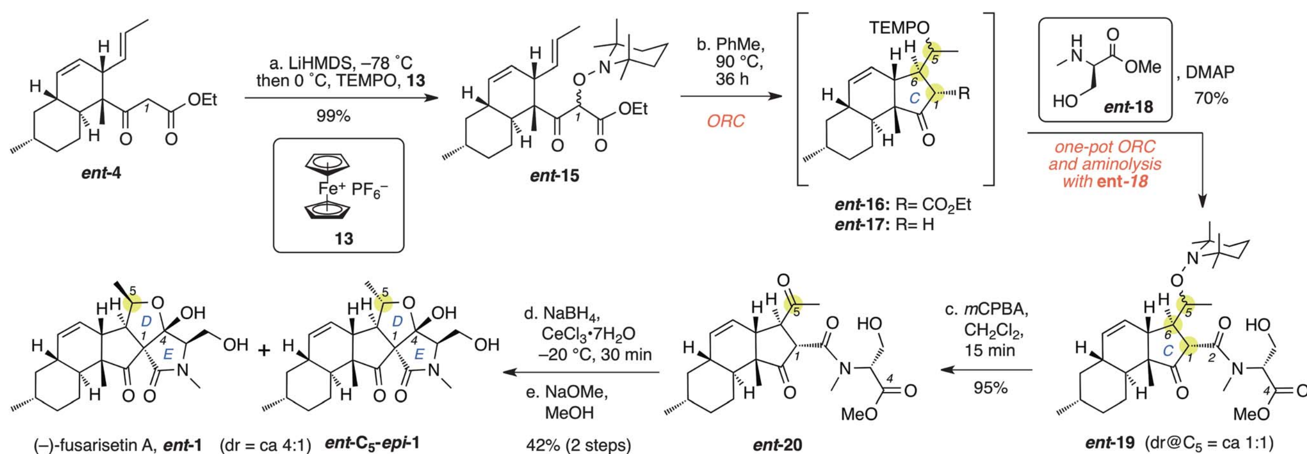

Scheme 2 First generation total synthesis of (−)-fusarisetin A (***ent*-1**) *via* a TEMPO-mediated ORC. *Reagents and conditions*: (a) LiHMDS (1.5 equiv.), 1,2-DME, −78 °C, 30 min, then 0 °C, TEMPO (1.05 equiv.), Cp_2FePF_6 (**13**, 2.0 equiv.), 5 min, 99%; (b) ***ent*-18**, DMAP (2.0 equiv.), PhMe, 4 Å MS, 90 °C, 36 h, 70% (dr = *ca.* 1 : 1); (c) *m*-CPBA (1.2 equiv.), CH_2Cl_2, 0 °C, 15 min, 95%; (d) $NaBH_4$ (1.2 equiv.), $CeCl_3 \cdot 7H_2O$ (1.5 equiv.), MeOH, −20 °C (dr = *ca.* 4 : 1); (e) NaOMe (5.0 equiv.), MeOH, 10 min, 42% for two steps.

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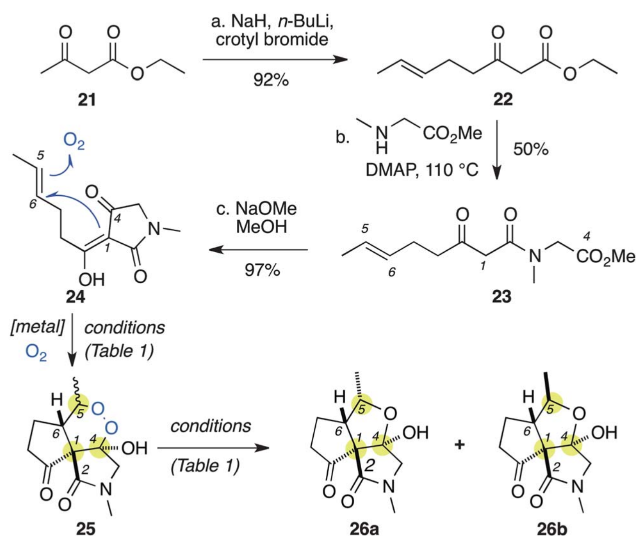
to compound ***ent*-19** (C_5 dr = *ca.* 1 : 1) in 70% overall yield. To avoid the difficult separation of these diastereomers, ***ent*-19** was directly treated with *m*-CPBA[39] to oxidatively cleave the N–O bond, producing ***ent*-20** in 95% yield. Regio- and stereo-selective reduction of this compound under Luche conditions[14,40] followed by a one-pot Dieckmann condensation/hemiketalization yielded (−)-fusarisetin A (***ent*-1**) together with its C_5-epimer (dr = *ca.* 4 : 1, 42% over two steps). Synthetic (−)-fusarisetin A was identical in all aspects with naturally occurring fusarisetin A (^{1}H, ^{13}C NMR and HR-MS), except for the optical rotation (synthetic: $[\alpha]_D^{23} = -86.2$ ($c = 0.065$ in MeOH); natural: $[\alpha]_D^{25} = +84.6$ ($c = 0.2$ in MeOH),[13] reported synthetic (−)-**1**: $[\alpha]_D^{27} = -88.0$ ($c = 0.15$ in MeOH).[14] The structure of ***ent*-C$_5$-*epi*-1** was confirmed by comparison to the literature data.[14,16]

Model studies on the metal-promoted ORC

Despite the overall efficiency of the one-pot TEMPO-mediated ORC/aminolysis reaction, the scale-up process suffers from significant decarboxylation of β-ketoester ***ent*-16**. Faced with this challenge, we evaluated alternative metal-promoted ORC reactions. To this end, we synthesized model system **24** that contains all key carbons needed for the proposed cyclization (Scheme 3). This compound was prepared *via* a sequence of three steps that included: (a) kinetic alkylation[41] of ethyl acetoacetate (**21**) with crotyl bromide to form **22**; (b) aminolysis of the ester group with *N*-methyl glycine methyl ester to produce **23**; and (c) Dieckmann condensation (formation of the C_1–C_4 bond) to yield **24** (45% yield overall). Tetramic acid **24** was then subjected to various reagents and conditions in order to perform the desired ORC (Table 1). Scarce literature reports[33] indicate the feasibility of this transformation that, nonetheless, has never been applied to intramolecular systems or any natural product synthesis. It is known that certain high oxidation state metals, such as Mn(III), Co(II), Ag(II), Pd(II), Pb(IV) and Ce(III), can promote the addition of carbon radicals derived from ketones to alkenes.[20,42] On the other hand, molecular oxygen exists as a persistent triplet diradical in its ground state and as such it can react rapidly with carbon-centered radicals.[43] With this in mind, we treated **24** with various metals in the presence of O_2 and, in certain cases, were able to isolate peroxyhemiketal **25** (as C_5 isomers). Reduction of the peroxide motif of **25** then produced **26** (as C_5 isomers) representing the tricyclic core of fusarisetin A.

The nature of the metal oxidant, oxygen pressure and reaction solvent/time were screened in order to optimize this ORC. Initial encouraging results were obtained using cobalt(II) acetate/AcOH/O_2 (1 bar) and afforded the desired *cis*-peroxyhemiketal **25** as a mixture of C_5 diastereomers (ratio 2 : 1) albeit in low yield (20%). Reduction of the cyclic peroxide group of **25** with CuCl yielded the corresponding tricyclic compounds **26a** and **26b** (ratio 2 : 1) in 80% overall yield (Table 1, entry 1).[44] Lower



Scheme 3 Model studies of metal-promoted ORC reactions. *Reagents and conditions*: (a) NaH (1.1 equiv.), THF, 0 °C, 10 min; then *n*-BuLi (1.05 equiv.), 0 °C, 10 min; crotyl bromide (1.07 equiv.), 0 °C to RT, 2 h, 92%; (b) *N*-methyl glycine methyl ester·HCl (2.0 equiv.), DMAP (2.0 equiv.), Et_3N (3.0 equiv.), PhMe, 110 °C, 12 h, 50%; (c) NaOMe (4.0 equiv.), MeOH, 2 h, 97%.

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Table 1 Selected conditions for the conversion of **24** to **25** and **26** *via* a metal-promoted ORC reaction[a,b]

Entry	Oxidant	T/°C	Time	Yield of **25** (%)	Reductant	Yield of **26** (%)
1	$Co(OAc)_2$	70	5 min	20	CuCl	80
2	$Co(OAc)_2$	70	5 min	20	Thiourea	n.r.[c]
3	$Co(OAc)_2$	25	4 h	10	CuCl	79
4	$Co(OAc)_2$[d]	25	12 h	n.r.	—	—
5	$CoCl_2$	25	12 h	n.r.	—	—
6	$Mn(OAc)_3$	25	12 h	5	—	—
7	$CeCl_3$	25	12 h	Trace	—	—
8	Fe(III)[e]	25	12 h	15	CuCl	81
9	CAN	25	3 h	57	CuCl	82
10	CAN	−20	18 h	57	CuCl	79
11	CAN	70	5 min	20	—	—
12	CAN[f]	25	3 h	40	CuCl	80
13	CAN[g]	70	3 h	30	CuCl	81

[a] For a detailed screening study see ESI.† [b] All the reactions were performed under 1 bar of oxygen and 1 equiv. of the oxidant in AcOH unless otherwise noted; yields of **25** and **26** refer to isolated products. [c] No reaction occurred. [d] *i*PrOH was used as the solvent. [e] Ferrocenium hexafluorophosphate. [f] 0.1 equiv. of CAN was used. [g] Reaction opened to air. CAN = cerium(IV) ammonium nitrate.

reaction temperature or replacement of the solvent/reductant led to unsatisfactory results (Table 1, entries 2–4). More than 20 metal or non-metal based oxidants were then further screened (see ESI†) under $AcOH/O_2$ conditions. Mn(III)-, Fe(III)- and Ce(III)-based oxidations were found to be inefficient for the conversion of **24** to **25** (Table 1, entries 6–8). Gratifyingly, cerium(IV) ammonium nitrate (CAN) significantly increased the yield of **25** to 57% (Table 1, entry 9). Decreasing the reaction temperature did not affect the diastereoselectivity at C_5 (Table 1, entry 10), while higher temperatures decreased the yield (Table 1, entry 11). Air or catalytic amounts of CAN (10 mol%) can also be used for the conversion of **24** to **25** but in this case the formation of **25** is low yielding (Table 1, entries 12–13). Nonetheless, in all cases the reaction proceeded in a highly stereocontrolled manner affording only two of eight possible diastereomers (ratio *ca.* 1.5 : 1 to 2 : 1).[44] It is worth mentioning that, independently of this study, Gao *et al* recently reported the conversion of (−)-equisetin to (+)-fusarisetin A using $Mn(III)/O_2$ conditions.[16]

Total synthesis of (−)-equisetin (2) and (+)-fusarisetin A (1)

Encouraged with the above results, we proceeded to implement the optimized ORC conditions to the synthesis of (+)-fusarisetin A using (−)-equisetin as the key synthetic intermediate (Scheme 4). Aldehyde **9** was rapidly and stereoselectively constructed from (*R*)-citronellal (**6**) in decagram-scale (35% over three steps) following the above procedure. Conversion of **9** to **4** proceeded under Reformatsky conditions followed by oxidation of the resulting alcohol with Dess–Martin periodinane (two steps, 92% overall yield, *ca.* 7 g prepared). It is worth noting that at this scale the IBX oxidation produced significant amounts of decarboxylated material. Aminolysis of ester **4** under various conditions, such as DMAP[14,15] and NHC-based reagents,[45] with *N*-methyl serine methyl ester (**18**)[46] afforded **11** in low yield. However, mild hydrolysis of **4** with ethanolic KOH (rt, 96 h) quantitatively produced the corresponding carboxylic acid that, upon coupling with **18** under HATU conditions afforded the desired amide **11** in 90% yield. Dieckmann condensation of **11** quantitatively produced a mixture of (−)-equisetin (**2**) and C_3-*epi*-equisetin (**C_3-*epi*-2**) (100%, dr = *ca.* 1 : 1).[16,17,24,31]

Gratifyingly, the previously defined ORC conditions were successfully applied for the conversion of (−)-equisetin (**2**) to (+)-fusarisetin A (**1**). Specifically, oxidation of **2** under $CAN/AcOH/O_2$ conditions produced an inseparable mixture of peroxy-fusarisetin A (**27**) and its C_5 epimer (**C_5-*epi*-27**) (dr = 1.3 : 1). This mixture was further reduced with thiourea (10 equiv.) to afford (+)-fusarisetin A (**1**) together with its C_5 epimer (**C_5-*epi*-1**) (62% overall, dr = 1.3 : 1).[47] The structures of both C_5 epimers of **27** and **1** have been confirmed by ^1H, ^{13}C NMR and HR-MS analysis.[13–16] As observed previously, the stereoselectivity of this ORC reaction is substrate-controlled and affords the desired stereochemistry at the C_1 and C_6 centers.

We were also able to use the mixture of equisetin (**2**) and C_3-*epi*-equisetin (**C_3-*epi*-2**) in the ORC reaction. In fact, 1.1 g of this mixture (dr = 1 : 1) were treated under $CAN/AcOH/O_2$ conditions and the resulting crude mixture of peroxy-fusarisetins was reduced with excess thiourea. Purification of this mixture produced 200 mg of (+)-fusarisetin A (**1**). Notably, the whole synthetic process from decalin **9** to (+)-fusarisetin A was performed on gram-scale and requires only one purification *via* column chromatography. In summary, the syntheses of both **1** and **2** are scalable, redox-/step-economic and protecting-group free.[48]

Biological studies of 1, 2 and related structures

To confirm and expand upon the previously reported findings, we evaluated the biological activity of (+)-fusarisetin A (**1**) in a scratch-wound assay and in a Boyden Chamber Transwell assay. The first assay (Fig. 2) involves inflicting a scratch wound in a confluent cell monolayer and measuring the migration of cells.[49] We were pleased to find that synthetic **1** inhibited migration of these cells at concentrations as low as 1 μg mL^{-1} (Fig. 2C) as compared to vehicle control (Fig. 2B). Importantly, removing **1** from the cells followed by incubation with fresh growth media allowed cells to migrate in a similar fashion to the control experiment (Fig. 2D). This observation demonstrates that the effect of **1** on these cells is reversible, in turn suggesting that (+)-fusarisetin A has little to no cytotoxicity at 1 μg mL^{-1} concentration.

We then performed Transwell migration assays using increasing concentrations of **1** normalized with the appropriate DMSO controls. This assay measures the capacity of cells to migrate across a porous membrane using serum-rich media as a chemo-attractant.[50] As seen in Fig. 3, cell migration was significantly inhibited at 3.0 and 6.0 μg mL^{-1}, while almost complete inhibition is observed at 12.0 μg mL^{-1}. Even at this concentration we did not observe any changes in the cell morphology, suggesting that **1** exhibits low cytotoxicity. The results of these two assays confirm the reported biological activity of fusarisetin A *in vitro*.

Encouraged by these findings, we then evaluated (+)-fusarisetin A in an *ex vivo* assay measuring migration of cells from a 5 mm mouse skin biopsy (Fig. 4).[51] We observed that both

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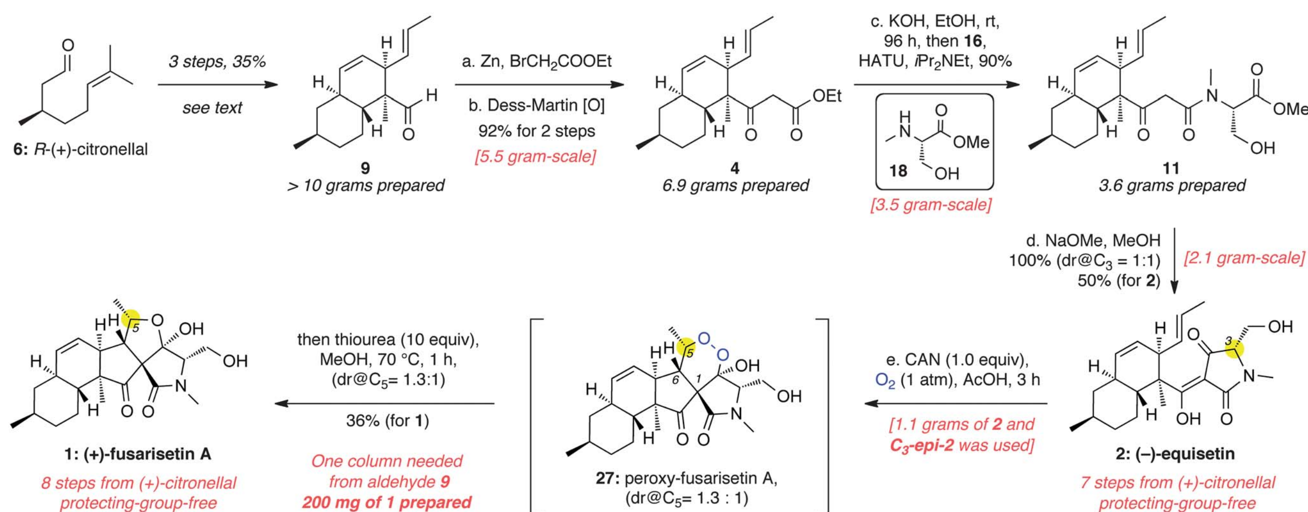

Scheme 4 Scalable total synthesis of (−)-equisetin (**2**) and (+)-fusarisetin A (**1**). *Reagents and conditions*: (a) activated zinc dust (5.0 equiv.), ethyl bromoacetate (3.0 equiv.), PhH, 90 °C, 45 min; (b) Dess–Martin periodinane (2.0 equiv.), CH_2Cl_2, RT, 2 h, 92% for two steps; (c) KOH (5.6 equiv.), EtOH, RT, 96 h, then **18** (1.2 equiv.), HATU (1.1 equiv.), CH_2Cl_2, DMF, 0 °C, *i*Pr_2NEt (3.0 equiv.), then RT, 2 h, 90%; (d) NaOMe (5.0 equiv.), MeOH, RT, 10 min, 100% combined yield (dr = 1 : 1); (e) CAN (1.0 equiv.), AcOH, O_2, RT, 3 h, then silica pad, thiourea (10 equiv.), MeOH, 70 °C, 1 h, 62% combined yield (dr = 1.3 : 1).

keratinocyte and fibroblast migration is inhibited upon exposure to **1** (10 μg mL^{-1}). Specifically, a substantial amount of cell migration is observed 5 days after plating the skin explant (Fig. 4B), as compared to the initial time of plating (Fig. 4A). In contrast, when explants were exposed to 10 μg mL^{-1} of **1** (Fig. 4C), there was no detectable migration of keratinocytes from the explants, while fibroblast migration was reduced by approximately 80%. The observed ability of fusarisetin A to inhibit cell migration from skin explants is particularly exciting. The migration of fibroblasts is an example of mesenchymal cell migration whereas keratinocytes move *via* collective cell migration.[52] Interestingly, compounds that target one type of migration have had disappointing results in clinical studies as the cancer cells are able to adapt and switch between different modes of migration.[53] Since fusarisetin A can significantly inhibit both types of migratory behavior, it could provide a powerful tool to circumvent the ability of cancer cells to alter their mode of motility if one pathway is inhibited.

Having demonstrated the cell-migration inhibitory properties of **1** both *in vitro* and *ex vivo* we then screened selected compounds containing the fusarisetin framework using the

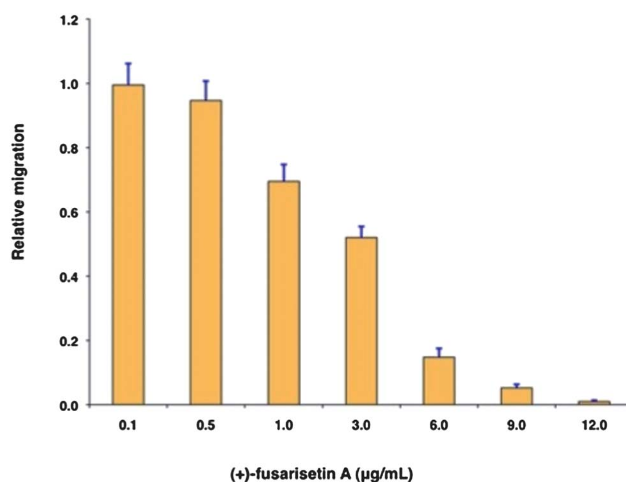

Fig. 3 (+)-Fusarisetin A (**1**) inhibits the migration of MDA-MB-231 breast cancer cells in a Transwell migration assay. Cells were plated in a Boyden chamber with **1** or vehicle control (DMSO) for 24 h at the indicated concentrations. Amount of migrated cells is presented relative to the corresponding DMSO control.

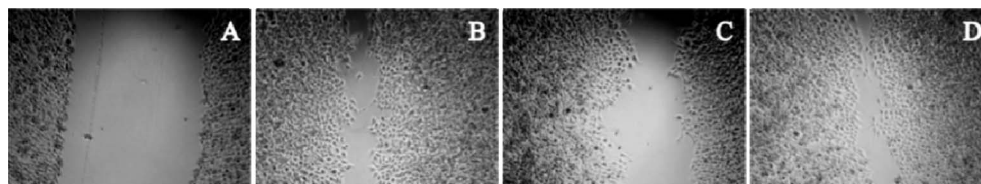

Fig. 2 (+)-Fusarisetin A (**1**) reversibly inhibits the migration of MDA-MB-231 breast cancer cells in an *in vitro* scratch-wound assay. Cells grown as a confluent monolayer were scratched, photographed, and incubated with DMSO (control) and **1** (1 μg mL^{-1} in DMSO). Micrographs (10× magnification) are presented showing cell migration across the scratch. (A): wound created at the time of the scratch (time = 0 h); (B) cell migration in presence of DMSO (time = 48 h); (C) cell migration in the presence of **1** (1 μg mL^{-1}), (time = 48 h); (D) wounded cells in panel C were rinsed with PBS to remove **1** and then incubated in fresh growth media for an additional 24 h.

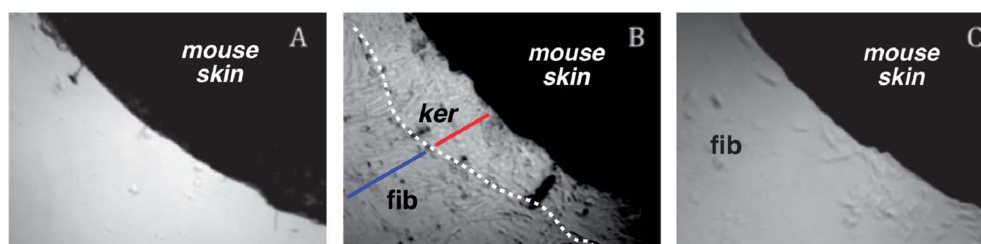

Fig. 4 (+)-Fusarisetin A (**1**) inhibits cell migration in an *ex vivo* mouse skin assay. Ker: keratinocyte migration (red line); fib: fibroblast migration (blue line). (A) 5 mm explant of mouse skin in a tissue culture dish (time = 0 days); (B) migration of cells from skin explant incubated for 5 days in growth media containing control amount of DMSO; (C) migration of cells from skin explant incubated for 5 days in growth media containing 10 μg mL^{-1} of fusarisetin A. The blue dotted line in (B) shows the migration area of keratinocytes. Solid lines indicate migration distance.

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scratch-wound assay.[54] We observed that natural (−)-equisetin (**2**) and its enantiomer ***ent*-2** do not exhibit any activity at the concentrations tested (Fig. 5). Similarly, *ent*-fusarisetin A (***ent*-1**) was found to be inactive. However, the C_5 epimer of natural fusarisetin A (**C_5-*epi*-1**) was found to display similar activity to that of (+)-**1**. These initial findings attest to the importance of the CDE ring structure and suggest that only the naturally occurring enantiomer of fusarisetin A could be used as a motif for the identification of new inhibitors of cell migration.

Conclusions

We report here a concise, efficient, and protecting group-free synthesis of fusarisetin A (**1**). Key to our synthetic strategy is the implementation of a bioinspired oxidative radical cyclization (ORC) reaction that forms the C ring of **1** *via* stereoselective construction of the C_1–C_6 bond. Subsequent oxidation at the C_5 center allows formation of the D ring of **1** ultimately converting equisetin (**2**) to fusarisetin A (**1**). The TEMPO-mediated ORC reaction could be successfully applied for the conversion of β-ketoester **4** to a tricyclic motif **19** that, upon Dieckmann condensation/hemiketalization, formed fusarisetin (**1**) together with its C_5 epimer (***C_5-epi*-1**). However, treatment of equisetin under these conditions produced exclusively the C_5 epimer of fusarisetin A (***C_5-epi*-1**). On the other hand, metal-mediated ORC reactions, such as $Co(OAc)_2$, $Mn(OAc)_3$, ferrocenium- and cerium(IV)-salts, could be successfully applied in a model system for the construction of the CDE ring of **1**. Moreover, the conversion of **2** to **1** was best achieved using CAN/AcOH/O_2.

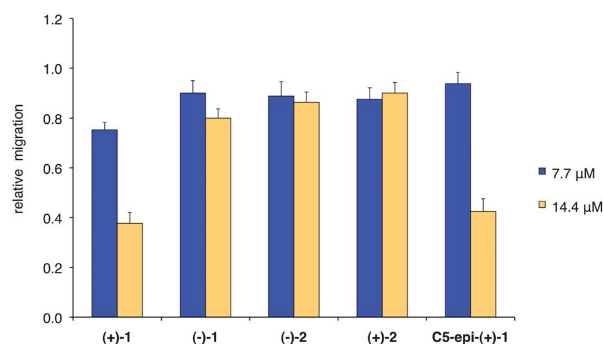

Fig. 5 Screening of fusarisetin A (**1**), equisetin (**2**) and selected synthetic intermediates in a scratch-wound assay at two different concentrations (7.7 and 15.4 μM). Values are given as relative migration in comparison to DMSO control.

Overall, the optimized synthesis of (+)-fusarisetin A proceeds in eight steps and about 5% yield starting from commercially available *R*-(+)-citronellal. We have also confirmed that (+)-fusarisetin A exhibits potent inhibitory activities against cancer metastasis *in vitro* and demonstrated its capability to inhibit different types of cell migration in mice skin. Interestingly, equisetin (either enantiomer) and (−)-fusarisetin A were found to be inactive in these assays, while ***C_5-epi*-1** displayed comparable activities to that of the natural product. In turn, this suggests that the motif of (+)-fusarisetin A could lead to new potent cancer metastasis inhibitors. The scalable synthetic strategy presented here could pave the way for more detailed structure–activity relationship and chemical biology studies.

Acknowledgements

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