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Synthesis and evaluation of structural requirements for antifungal activity of cyrmenin B_1 analogues

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ABSTRACT

In a study aiming to define the structural elements essential to cyrmenin B_1 antifungal activity, the synthesis of a series of analogues was carried out. The structural modification introduced stepwise in distinct areas of the parent molecule allowed a deeper insight to be gained into the most relevant structural features affecting the activity of the natural compound. The bioassay results clearly indicate the relevance of each portion of the parent molecule, only the modification of the conjugated double bond geometry being tolerated.

Chemical crop protection has become an essential tool to guarantee product yields and quality and to meet the ever-growing demands of humans.[1]

Nature has been a very fruitful source of new compounds and natural substances still exert a strong influence on modern crop protection development, by serving as lead structures for the discovery of active molecules, often featuring novel and unique modes of action.[2]

Promising biological activity and broad spectrum against different plant pathogens promoted the choice of fungicides originating from microbial sources. However, microbial fungicides have well known limitations in their development and practical use. Disadvantages are for example the low productivity of fungicidal metabolites by microorganisms when compared to the yields of synthetic chemicals, and often the low stability in the conditions of use in agriculture.[3]

Recently, Sasse et al.[4] have isolated novel antifungal metabolites, named cyrmenin A, B_1 and B_2 (Fig. 1), from the culture broth of strains of the myxobacteria *Cystobacter armeniaca* and *Archangium gephyra*.

The cyrmenins are modified *N*-acyldipeptide esters containing a dehydroalanine, a 2-amino-3-methoxyacrylate moiety, and a (2*E*,4*Z*)-undecadienoic or dodecadienoic acid residue. With their two adjacent dehydroaminoacids and an N-substituted 2-amino-3-methoxyacrylate unit, the cyrmenins represent a unique series of natural products. They exhibit high antifungal activity, showing at the same time an exceptionally low toxicity for animal cell cultures.[4a]

In a previous paper we reported the first total synthesis of cyrmenin B_1 (**9a**) and of its (8*E*,10*E*)-geometrical isomer (**9b**).[5]

Due to its modular nature, the developed synthetic route allowed the preparation of some representative derivatives and analogues for structure–activity relationship studies. This Letter discusses the approaches to the optimization of the lead structure, to gain a deeper insight into the most relevant structural features affecting the antifungal activity of cyrmenin B_1.

Our strategy to modulate the activity focused on modifications in three distinct areas of the molecule: the lipophilic unsaturated

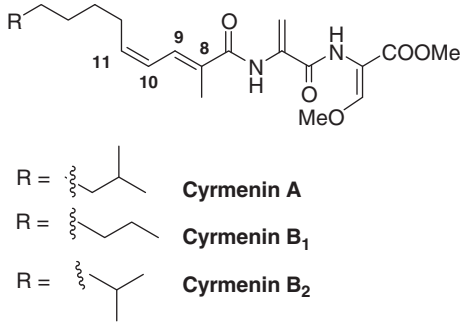

Figure 1. Structures of the cyrmenins.

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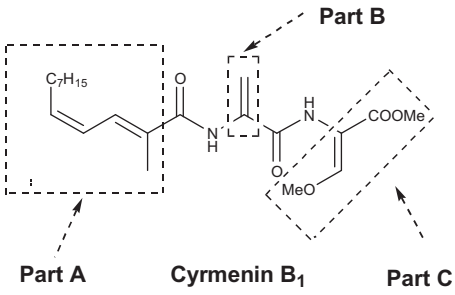


Figure 2. Regions of cyrmenin B_1 modified in the synthetic analogues.

chain (Part A, Fig. 2), the dehydroalanine moiety (Part B) and the β-methoxyacrylate system (Part C).

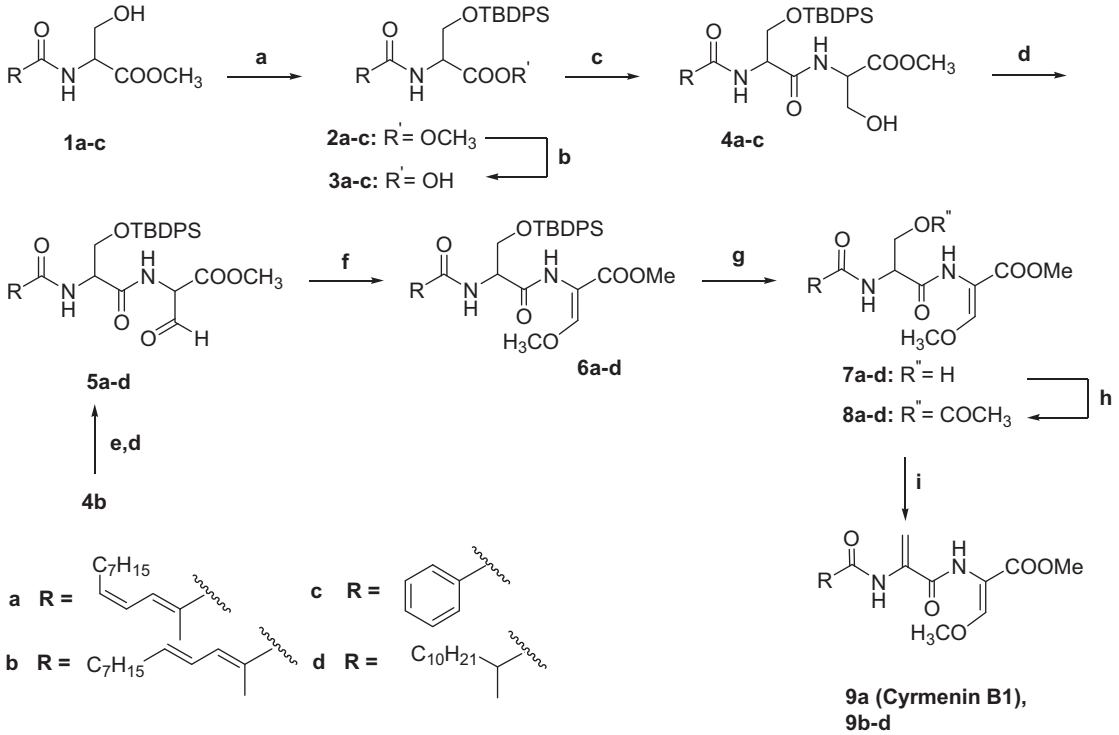
To elucidate the role of the conjugated (8*E*,10*Z*) double bonds (Part A), we prepared and tested, besides the natural compound **9a**, the unnatural cyrmenin (**9b**) with (8*E*,10*E*) double bonds. The synthetic pathway, summarised in Scheme 1, started from *N*-acyl-serine derivatives **1a** and **1b**, prepared from (2*E*,4*Z*)-2-methyldodeca-2,4-dienoic acid and (2*E*,4*E*)-2-methyldodeca-2,4-dienoic acid.[5] Exploiting the same strategy, we prepared compounds **9c** and **9d** bearing a phenyl ring and a saturated chain in the left hand side, respectively. The synthesis of **9c** started from benzoylserine methyl ester **1c**.[6] The synthesis of **9d** entailed the hydrogenation of the *N*-acyl dipeptide alcohol **4b** followed by oxidation of the product by treatment with IBX in ethyl acetate at reflux, to give the desired aldehyde **5d**. The required β-methoxyacrylate group was inserted while reacting **5a**–**d**, as a tautomeric mixture of keto/enol forms, with $TMSCHN_2$ in methanol/toluene.[7] Deprotection of the silyl ether by treatment with TBAF and acetylation gave the dipeptide alcohol **7a**–**d** in high yield. The dehydroaminoacid moiety was then obtained by the treatment of acetyl derivatives **8a**–**d** with the weak Lewis acid $LiClO_4$ and DBU at −15 °C.[8] Compounds **9c** and **9d** were obtained in moderate yields (50–55%).

Concerning Part B modifications, we prepared compound **14** with an alanine moiety in place of the dehydroserine found in the natural cyrmenin, assuming that its removal could possibly give advantages such as increased metabolic stability (Scheme 2). L-Alanine methyl ester hydrochloride was coupled with (2*E*,4*Z*)-2-methyldodeca-2,4-dienoic acid **10** in the presence of BOP and DIPEA to afford the amide ester **11** that was quantitatively converted into acid **12** by alkaline hydrolysis. Subsequent coupling with D,L-serine methyl ester hydrochloride furnished the dipeptide alcohol **13**. IBX oxidation in ethyl acetate gave an unstable aldehyde, which was immediately converted into β-methoxyacrylate **14** by treatment with $TMSCHN_2$.

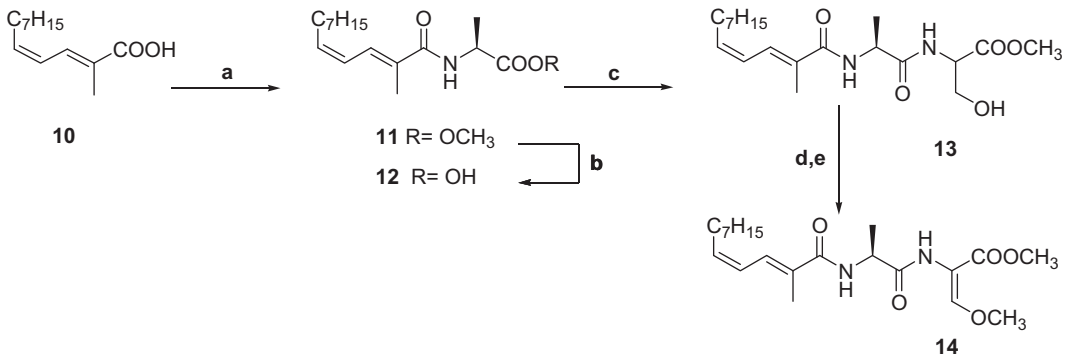
Compounds **7a** and **7b** (Scheme 1), intermediates in the synthesis of cyrmenin B_1 and its geometrical isomer **9b,** respectively, can be considered as cyrmenin analogues modified at the central Part B, as they bear a hydroxymethyl group in place of the *exo* double bond.

Finally, we tried to verify if any antifungal activity could be retained by cyrmenin derivatives lacking the methoxyacrylate moiety (Part C modifications). The synthesis was achieved following the pathway outlined in Scheme 3. Condensation of compound **1a** with two units of D,L-serine methylester hydrochloride afforded compound **16** that, after double acetylation and elimination, gave compound **18** in a 52% yield.

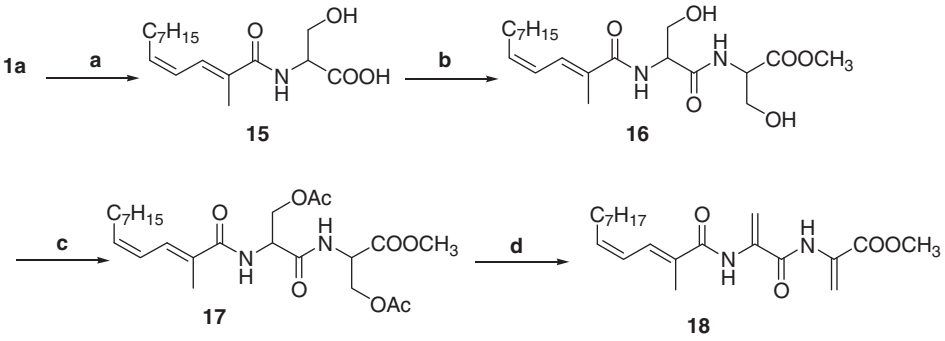
The activity of cyrmenin B_1 (**9a**), and of 7 analogues (**9b–d**, **14**, **7a–b**, **18**) was tested against *Bacillus subtilis* (Ehrenberg) Cohn, strain IPV 2430, *Saccharomyces cerevisiae* Meyen ex Ec. Hansen, strain IPV 637, *Aspergillus niger* Tegh., strain IPV F303, *Botrytis cinerea* Pers., strain IPV F5.2, *Cochliobolus miyabeanus*, (S.Ito and Kurib) Drechsler ex Dastur, and *Pyricularia oryzae* Cavara, strain IPV A1, according to the method reported by Sasse et al.[4a]



Scheme 1. Synthesis of compounds **9a**–**d**. Reagents and conditions: (a) TBDPSCl, imidazole, CH_2Cl_2, 0 °C, 95–97%; (b) LiOH, THF/H_2O; (c) D,L-serine methyl ester hydrochloride, BOP, iPr_2EtN, THF, 0 °C to rt, 53–76%; (d) IBX, EtOAc, reflux; (e) Pd/C (10%), EtOAc, 24 h, rt; (f)$TMSCHN_2$, toluene/methanol, rt, 24–65%; (g) TBAF, THF, 0 °C to rt, 54–75%; (h) AcCl, Py, CH_2Cl_2, 0 °C to rt, 88–97%; (i) $LiClO_4$, DBU, THF, −15 °C to rt, 50–58%.



Scheme 2. Synthesis of alanine derivative **14**. Reagents and conditions: (a) L-Alanine methyl ester hydrochloride, BOP,*i*Pr$_2$NEt,THF, 0 °C to rt, 3h, 95%; (b) LiOH·H$_2$O, THF/H$_2$O, 1 h; (c) D,L-serine methyl ester hydrochloride, BOP, *i*Pr$_2$NEt,THF, 0 °C to rt, 3 h, 75%; (d) IBX, EtOAc, reflux, 7 h; (e) TMSCHN$_2$, CH$_3$OH/H$_2$O, rt, 2 h, 66%.



Scheme 3. Synthesis of derivative **18**. Reagents and conditions: (a) LiOH, THF/H$_2$O; (b) D,L-serine methyl ester hydrochloride, BOP, HOBT, THF, 53%; (c) acetyl chloride, pyridine, CH$_2$Cl$_2$, 85%; (d) LiClO$_4$, DBU, THF, 52%.

The data confirmed the activity of cyrmenin B$_1$ against some filamentous fungi such as *Pyricularia* and *Aspergillus* sp. However, in our experiments we did not observe any activity of cyrmenin B$_1$ towards *S. cerevisiae* and *B. cinerea*. The biological data regarding the analogues featured by modifications at Part A highlight that *Z* geometry of the double bond at position 10 is more favourable for the activity than the *E* geometry. Moreover, the replacement of the conjugated double bonds with the corresponding saturated chain (**9a**, **9b** vs **9d**) caused a complete loss of antifungal activity. Compound **9c**, bearing a phenyl group in place of the (2*E*,4*Z*)-dodecadienoic residue, was inactive as well. These results underline that the conjugated diene, preferably with (8*E*,10*Z*) geometry, is an essential moiety.

The modifications introduced at the *exo* double bond of the parent molecule (Part B) demonstrate that also this element is crucial for the antifungal activity. In fact, both compounds **14**, bearing an alanine residue, and **7a** and **7b**, bearing a serine, were inactive against all the tested strains.

The replacement of the methoxy group in the β-methoxyacrylate group with a hydrogen atom (compound **18**) again resulted in a complete loss of antifungal activity.

Collectively, the cyrmenin B$_1$ analogues, except for the geometrical isomer **9b**, showed a complete lack of antifungal activity. Thus, only **9a** and **9b** were further investigated.

The growth inhibition of *A. niger* and *P. oryzae* induced by these compounds was assayed by the commonly used 'poisoned food' technique. The activity at four different concentrations is reported in Table 1 as percent inhibition of mycelial growth in comparison to the untreated control (For experimental details see Supplementary data).

Table 1
Percent inhibition of mycelial growth induced by compounds **9a** and **9b**

Concn μgm L^{-1}	*Aspergillus niger*[a]		*Pyricularia oryzae*[b]	
	9a	**9b**	**9a**	**9b**
25	5	0	32	28
50	5	5	40	31
100	11	9	52	40
200	27	11	65	44

[a] Standard compound tetraconazole, 100% inhibition at 20 mg L^{-1}.
[b] Standard compound azoxystrobin 100% inhibition at 100 mg L^{-1}.

The data showed that cyrmenin B$_1$ (**9a**) is effective for the reduction of *P. oryzae* mycelial growth, with the reduction of sporulation, while its effect against *A. niger* at the same concentration was moderate. Compound **9b** showed activity against both fungi even if lower than the activity showed by the parent molecule.

In conclusion, a series of cyrmenin B$_1$ analogues were synthesized and assayed for antifungal activity and structure–activity relationship studies. Only the modification of the conjugated double bond geometry (**9a** vs **9b**) was tolerated. The complete lack of activity of compounds **9c** and **9d** underlines that simply maintaining the lipophilicity of portion A in the molecule is not enough to have active compounds. Replacement of the *exo* methylene group with a simple methyl group was also detrimental. The modification

of the expectedly crucial (compare strobilurins[9] and analogues) β-methoxyacrylate group was also deleterious.

The bioassay results clearly indicate the relevance of each portion of the parent molecule, leaving very little space for synthetic modifications. This kind of issue could represent a limitation for the future development of new fungicidal compounds deriving from natural cyrmenins.

Acknowledgments

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Supplementary data

Supplementary data (experimental procedure and spectral data for all compounds) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.11.023.

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