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SAR Studies of the Leupyrrins: Design and Total Synthesis of Highly Potent Simplified Leupylogs

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Abstract: Leupyrrins are highly potent antifungal agents. A structure-activity-relationship study of natural and synthetic derivatives is reported which reveals important insights into the biological relevance of several structural subunits leading to the discovery of highly potent but drastically simplified leupylogs that incorporate a stable and readily available aromatic side chain. For their synthesis a concise strategy is described that enables a short and versatile access.

The leupyrrins present structurally complex macrodiolides from Sorangium cellulosum.^[1] As shown for leupyrrins A_1 (1) and B_1 (2, Figure 1) they are characterized by an 18 membered nonsymmetric cyclic core with an unusually substituted *p*-butyrolactone ring together with a pyrrole and an oxazoline in combination with a unique dihydrofuran side chain containing two exocyclic alkylidenes. They exhibit not only very potent antifungal activities but also antiproliferative and anti-HIVproperties.^[1, 2] So far the drug target has not been identified but conventional targets are not addressed, which may suggest that an unusual biochemical interaction site(s) may be involved.^[1] However, the further exploration has been severely hampered by the low availability, in combination with their pronounced structural complexity and a lability of the diene residue in the side chain that is prone to double bond shifts. We have recently isolated natural leupyrrins, determined their full stereochemistry and developed a modular synthetic strategy which culminated in the total synthesis of leupyrrins A₁ and B₁ (1 and 2, see Figure 1).^[3-5] Herein, we report first detailed SAR studies with five natural and six synthetic analogues that vary in the side chain, the furan, the oxazoline, the pyrrole moieties and the diacid fragment. Dramatically simplified leupylogs of type 3 and 4 were developed that are characterized by a simplified aromatic side chain and that retain potent biological activities (3).

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Figure 1. Leupyrrin A_1 (1) and structure related leupylogs 3 and 4. The structural modifications are highlighted in red.

Despite considerable progress in leupyrrin total synthesis,^[3-5] preparation of the *bis*-alkylidene substituted furane moiety has remained a challenge, due to the lability of this subunit and the high number of synthetic steps required, despite the advancement of a concise a one-pot process. Therefore, a prime focus of this study was placed on simplification and stabilization of this segment. As shown in Figure 1, it was envisioned that the alkyliden units may be stabilized by incorporation into an aromatic moiety. Furthermore, biological activity tolerance for side chain variants of natural leupyrrins (see below) also suggested that the aliphatic residue may also be simplified. In combination, this led to design of analogues **3** and **23** (Scheme 1).

For their synthesis, the basic strategy developed during leupyrrin total syntheses was applied, involving an sp²-sp³ cross coupling to forge the C6-C7 bond, a cyclodehydration for oxazolin condensation (C13-C14) in combination with an esterification (C4'-C19) and a final macrolactonisation (C1'-C3). For implementation of this approach, Eastern fragments 10 and 11 were required. They were obtained in a short approach from known epoxyalcohols $\mathbf{5}^{[6]}$ and $\mathbf{6}^{[7]}$ by coupling with diacid $\mathbf{7}^{[5]}$ using the Shiina protocol^[8] and regio- and stereoselective epoxide opening of derived esters 8 and 9 under Brønsted acidic conditions with NH4Cl and NaN3 as a nitrogen source (Scheme 1).^[9] Small amounts of likewise obtained C2-esters could be removed by chromatography. Subsequent azide reduction by hydrogenation in presence of Pd/C gave amino alcohols 12 and 13 which may be used without further purification. Alternatively, also a Staudinger reduction with polymer-bound triphenylphosphine may be applied, leading to quantitative yields for 12 (see SI section). For further elaboration, azabenzotriazole **16** was prepared from pyrrole **14** and γ -lactone 15 following our previously reported procedure,^[3-5] involving a

COMMUNICATION

well-scalable tandem sequence for **15**. Subsequent amide formation of **16** with either **12** and **13** under basic conditions followed by cyclodehydration with DAST^[10] gave oxazolines **17** and **18** in high yield (90 and 87%). For conversion to *seco*-acids **19** and **20**, cleavage of the *tert*-butyl esters was realized with TMSOTf/lutidine^[11] followed by selective acetate saponification using K₂CO₃ in a THF/MeOH/H₂O mixture. Macrolactonization was then realized by applying the Shiina protocol^[8] under high dilution conditions to give macrolactons **21** and **22**, and finally TASF mediated deprotection yielded the desired leupylogs **3** and **23**.



Scheme 1. Concise three-step synthesis of simplified Eastern building blocks 12 and 13 and completion of the synthesis of leupylogs 3 and 23.

Encouraged by the potent biological activities of these two analogues (see below), we opted for further modifications and studied the biological role of the pyrrole by replacing it with a furan and a thiophen, giving analogues **4** and **36**. As shown in Scheme 2, their synthesis was based on initial cross coupling of bromides **24** and **25**^[12] with **15**. While the conditions developed

leupyrrin synthesis^[5] involving Pd(P^tBu₃)₂, Cs₂CO₃ in for THF/H₂O were directly applicable to access 26 in 99% yield, coupling with thiophene 25 required adjustment of parameters. Finally, optimal results to obtain 27 in quantitative yield were realized with Pd(PPh₃)₄ in presence of K₃PO₄ in THF/water at 60 °C.^[13] Subsequently, removal of the tert-butyl ester with TMSOTf/lutidine,^[11] conversion of derived crude acids to active esters 28 and 29 and cyclodehydration $^{\![3]}$ with amine 12 gave oxazolines 30 and 31 in high yield. In contrast to previously reported route (Scheme 1), application of conditions for cleavage of tert-butyl ester (TMSOTf) and acetate esters (K₂CO₃) were unreliable or led to decomposition of **30** and **31**. After considerable attempts, a deprotection sequence incorporating magnesium methoxide^[14] for acetate cleavage at 0°C and zinc bromide^[15] in dichloromethane at room temperature for tert-butyl removal enabled a reliable access to seco-acids 32 and 33. Subsequent Shiina macrolactonization gave macrocycles 34 (73%) and 35 (94%) in a good and excellent yield. Finally, removal of the remaining primary TBSgroup proceeded smoothly by utilizing TBAF in THF at 0 °C to reveal leupylogs 4 in 96% and 36 in 61% yield, which proved more reliable as compared to TASF.



Scheme 2. Synthesis of Western fragments 26 and 27 and completion of the synthesis of leupylogs 4 and 34.

Inspired by a certain remaining activity of **4** in combination with a high biological tolerance of the diester fragment observed for

COMMUNICATION

natural leuyprrins, more truncated leupylog 51 (Scheme 3) was targeted, which contains an oxazol instead of the oxazoline and a simplified diacid building block. For its synthesis, a modified Northern building block (43) was required. Its synthesis was initiated by treatment of α -chloro- β -keto-ester **38**^[16] with a mixture of commercially available acid (37) and Hünig's base in acetonitrile^[17] to give β -keto-ester **39**. Subsequent microwave assisted heating in acetic acid in presence of ammonium trifluoroacetate $^{\left[18\right] }$ gave desired oxazole 40 together with imidazole 41, which could be removed by chromatography. After reduction of ester 40 with DIBAL-H, resulting alcohol was protected as acetate 42. Selective side chain bromination with NBS then gave furfuryl bromide 43 (80%) together with small amount of 44 (7%), derived from double bromination.^[19] This side product could be removed at a later stage of aldehyde stage (46, see SI section).



Scheme 3. Synthesis of Northern building block 43 and completion of the synthesis of leupylog 51.

The next step required a challenging sp^2-sp^3 cross coupling with boronate **15**. Gratifyingly, by applying our initially optimized conditions^[5] (see above), the desired product **45** could be isolated in 58% yield. For introduction of symmetric diacid a one-pot esterification-macrolactonization was then evaluated, requiring diol **47**. Subsequent saponification studies to access **47** from **45** using K₂CO₃ in MeOH indicated the primary alcohol to be cleaved first, followed by slower liberation of the secondary hydroxyl group. However, as extended reaction times led to unexpected decompositions, it proved beneficial to keep them very short (6 minutes) and to stop the reaction

before full conversion by addition of saturated NH₄Cl. This strategy gave mainly desired diol **47** together with variable amounts of easily separable mono-deprotected **48**, which may be submitted to another round of ester cleavage. Gratifyingly, the pivotal one-pot macrolactonization of diol **47** could then be realized with succinic acid anhydride (**49**) applying the Shiina protocol,^[8] treating a highly diluted solution of anhydride **49**, MNBA and DMAP in dichloromethane very slowly over several hours with a solution of diole **47** giving the desired macrodiolide **50** in respectable yield (44%), considering the synthetic challenge of this tandem process. Finally, removal of the remaining TBS-group proceeded smoothly with TBAF buffered with AcOH to yield truncated leupylog **51**.

Biological assays

Antifungal activities were first assayed for natural leupyrrins **1**, **2**, **52–55** and triol **56** (Figure 2), which was obtained by saponification of leupyrrin B_1 (see SI-section).



Figure 2. Overview of natural leupyrrins 52–55 and triol 56.

As outlined in Table 1, all natural leupyrrins show similarly highly efficacious fungal cell growth inhibitory effects in the low μ g/mL range towards the yeast *Rhodotorula glutinis*, and the two molds *Botrytis cinerea* and *Mucor hiemalis* (both of which are important plant pathogens) in agreement with the data originally published for **1**.^[1] In addition, none of them showed activity against the yeast *Saccharomyces cerevisiae* or the Grampositive bacterium *Staphylococcus aureus*.

These data suggest that slight modifications of the diacid fragment and aliphatic side chain have only a minor effect on antifungal activity. On the other hand, triol **56** showed no activity against all tested bacterial and fungal organisms, revealing the whole macrocycle to be essential.

COMMUNICATION

Table 1. Biological activity of natural leupyrrins (1, 2, 52-55) and Triol 56.

	MIC [µg/mL]							
Compound	A ₁ ^[a] (1)	A ₂ (53)	B ₁ (2)	B ₂ (54)	C (52)	D (55)	Triol 56	
Rhodotorula glutinis	0.25	0.47	0.47	0.97	0.78	0.47	>40	
Botrytis cinerea	0.80	1.25	1.25	1.25	1.25	1.25	>40	
Mucor hiemalis	0.30	0.24	1.25	0.31	0.47	0.31	>40	
Saccharomyces cerevisiae	n.d.	>40	>40	>40	>40	>40	>40	
Staphylococcus aureus	n.d.	>40	>40	>40	>40	>40	>40	

^[a] Value from ref. [1].

We then evaluated antifungal activities of leupylogs 3, 4, 23, **36** and **51** in direct comparison to leupyrrins A_1 (1) and B_1 (2), as well as with Amphotericin B (AmB) as a control using the yeasts Candida albicans, a human pathogen, or Rhodotorula glutinis as test organisms. As shown in Table 2, aryl-substituted leupylogs 3 and 23 displayed potent IC₅₀ values in the same range as the parent natural products against R. glutinis, demonstrating that substitution of the dihydrofuran side chain by an aryl group is well-tolerated. In contrast, furan leupylog 4 was less active and the thiophene analogue 36 was essentially inactive, demonstrating that the original pyrrole may not be easily substituted. Notably, the pyrrole N-H of the original heterocycle exerts a characteristic hydrogen bond to the neighboring oxazoline, leading to an N,N-syn orientation of the two respective nitrogen atoms, which has a pronounced effect on the macrocyclic conformation in solution and solid state.^[3] Potentially, a hydrogen bond donor like the pyrrole is also be necessary for the correct conformation of the molecule at the target. The entire exchange of the pyrrole-oxazoline structure by a furan-oxazole unit as in leupylog 51 led to complete loss of activity. These data suggest that the pyrrole-oxazoline subunit and the macrocyclic ring constitute important parts of the pharmacophore.

Table 2. Biological activity of natural leupyrrin A_1 (1) and B_1 (2) and synthetic leupylogs 3, 4, 23, 36 and 51 with Amphotericin B (AmB) as a control.

	IC ₅₀ [μg/mL]									
Compound	1	2	3 2	34	36	51	AmB			
Rhodotorula glutinis	0.29	0.14	0.23 0.2	21 10	>16	>16	2.45			
Candida albicans	>64	>64	>64 >6	64 >16	>16	>64	0.33			
Mouse fibroblasts (L929)	0.95	2.09	9.82 5.0	08 >40	>40	5.69	n.d.			

Furthermore, none of the compounds tested were active against *C. albicans.* As **23** was the most active and potent leupylog, we evaluated it further and found it to be highly active against *R. minuta* and *R. mucilaginosa* and against *Zygosaccharomyces bailii* (MIC's of 1 μ g/ml and <0.125 μ g/ml, respectively). The growth of *Zygosaccharomyces bailii* was impaired, but not completely inhibited at concentrations up to 16 μ g/ml. As a control for unspecific toxicity, the yeast *Candida*

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glabrata that is resistant against natural leupyrrins, was also resistant to 23 (see SI section). While the insensitive organisms are all capable of fermenting glucose, members of the genus Rhodotorula possess a respiratory (non-fermentative) metabolism. These differences in metabolism might account for the variations in susceptibility observed. In order to assess whether leupylogs have a specific antifungal activity, or also affect the growth of mammalian cells, the natural leupyrrins 1 and 2 and synthetic leupylogs 3, 4, 23, 36 and 51 were tested in an antiproliferative assay with the murine fibroblast cell line L929. Natural leupyrrins 1 and 2 and antifungal leupylogs 3, 23 and 51 also exhibited antiproliferative potencies with similar IC₅₀ values, which indicates that the molecular target is likely to be evolutionary conserved for these simplified analogs. In contrast, leupylogs 4 and 36 were inactive, but also had poor antifungal activity.

In summary, a simplified series of leupyrrin analogues has been developed which are characterized by a drastically simplified aromatic side chain. A first set of relevant SAR data for the leupyrrins has been obtained with natural and synthetic derivatives, focusing on variations of the macrocyclic ring, the pyrrole and oxazoline fragment, the side chain and the diacid building block. They revealed a high importance of the macrocyclic core, a certain flexibility of the diacid fragment and high tolerance of the side chain. Two of these leupylogs retain the potent biological activity of the parent natural products. This demonstrates that the overall structure can be simplified, which is quite remarkable as only few such examples have been described in the context of complex natural macrolides.^[20] The most potent leupylogs 3 and 23 are characterized by a substitution of the synthetically challenging and chemically labile bis-alkylidene diene-substituted dihydrofuran side chain of the authentic leupyrrins by a mere aryl substituent, which also replace the fragment. These leupylogs are much more readily available as compared to the parent natural product (17 steps for 3 vs 24 steps for 1 in the longest linear sequences from commercial material), involving a much more readily available Eastern fragment (6 steps for 12 from commercial material vs. 18 steps for the authentic Eastern fragment of leupyrrin A₁). Furthermore, these studies suggest that the pyrrole-oxazoline subunit seems to be a crucial element of their pharmacophore, which may be rationalized by solution conformation considerations. Due to the facilitated practical access, many more leupylogs may now be envisioned, enabling more detailed biological studies of these potent antifungal agents. The increased availability and the potential modifications will aid the drug target identification and will help to evaluate practical applications in basic research and medicine.

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Entry for the Table of Contents

Novel simplified leupyrrins retaining powerful antifungal potencies were efficiently obtained by a concise sp^2-sp^3 cross coupling and macrolactonization.



Macrolides*

Paul R. Wosniok, Christopher Knopf, Sandra Dreisigacker, J. Manuel Orozco-Rodriguez, Bettina Hinkelmann, Peter P. Mueller, Mark Brönstrup, and Dirk Menche SAR Studies of the Leupyrrins: Design and Total Synthesis of Highly Potent Simplified Leupylogs