Natural Products | *Very Important Paper*

# 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 non-symmetric cyclic core with an unusually substituted $\gamma$-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-HIV-properties.[1,2] So far the drug target has not been identified but conventional drug targets are not affected, 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 $A_1$ and $B_1$ (**1** and **2**, see Figure 1).[3–5] Herein, we report the 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**).

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 stabiliza-

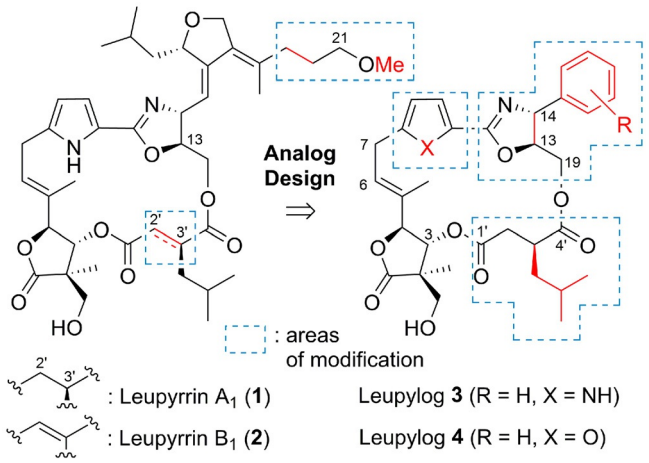


**Figure 1.** Leupyrrin $A_1$ (**1**) and structure related leupylogs **3** and **4**. The structural modifications are highlighted in red.

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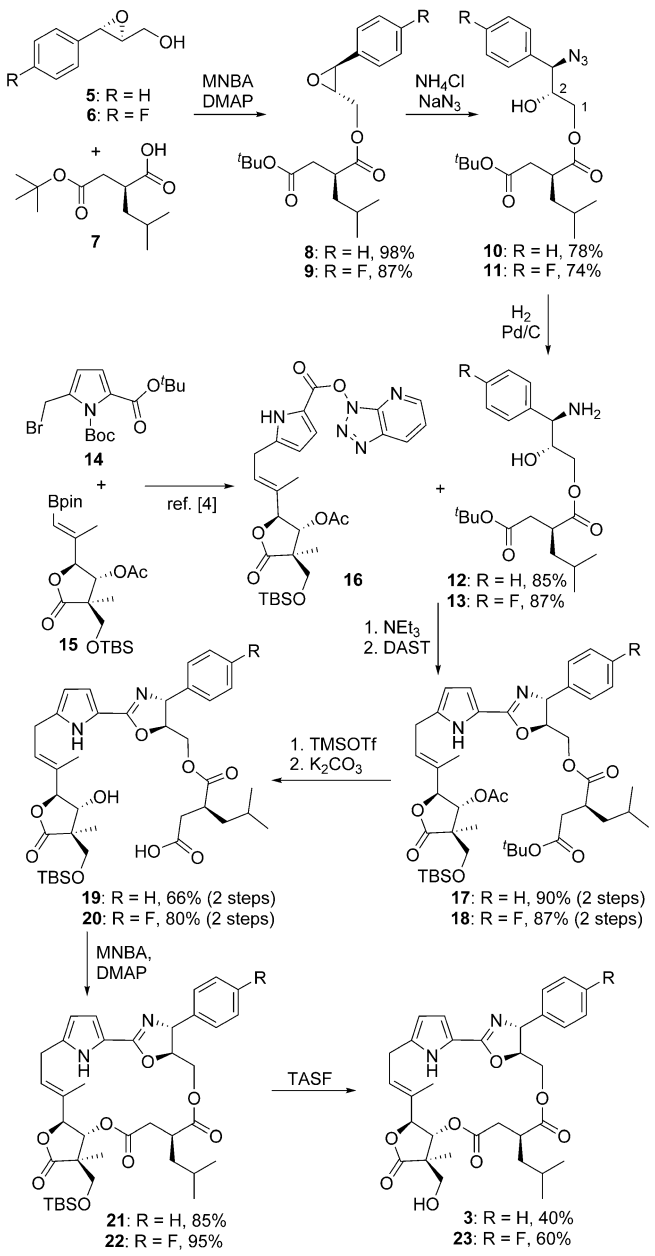
tion of this segment. As shown in Figure 1, it was envisioned that the alkyliden units may be stabilized by incorporation into an aromatic moiety. Furthermore, biological activity tolerance for side chain variants of natural leupyrrins (see below) also suggested that the aliphatic residue may also be simplified. In combination, this led to design of analogues **3** and **23** (Scheme 1).

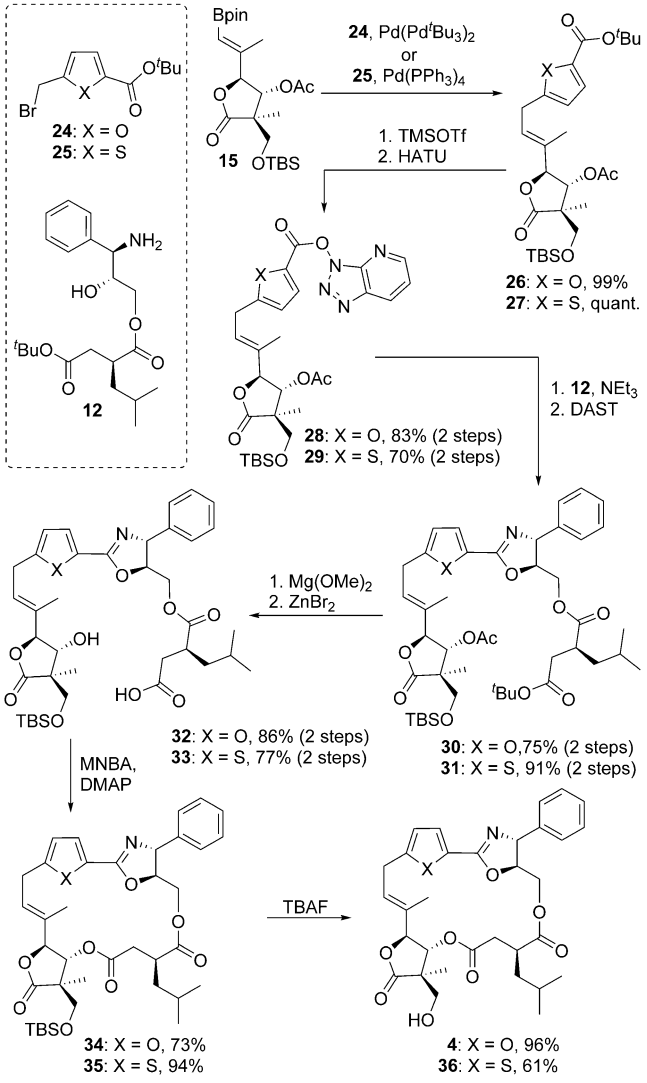
For their synthesis, the basic strategy developed during leupyrrin total syntheses was applied, involving an $sp^2$–$sp^3$ cross coupling to forge the C6–C7 bond, a cyclodehydration for oxazolin condensation (C13–C14) in combination with an esterification (C4′–C19) and a final macrolactonisation (C1′–C3). For implementation of this approach, Eastern fragments **10** and **11** were required. They were obtained in a short approach from known epoxyalcohols **5**[6] and **6**,[7] by coupling with diacid **7**[5] using the Shiina protocol[8] and regio- and stereoselective epoxide opening of derived esters **8** and **9** under Brønsted acidic conditions with $NH_4Cl$ and $NaN_3$ 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 the 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 Supporting Information). For further elaboration, azabenzotriazole **16** was prepared from pyrrole **14** and γ-lactone **15** following our previously reported procedure,[3–5] involving a 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 $K_2CO_3$ in a THF/MeOH/$H_2O$ 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**.



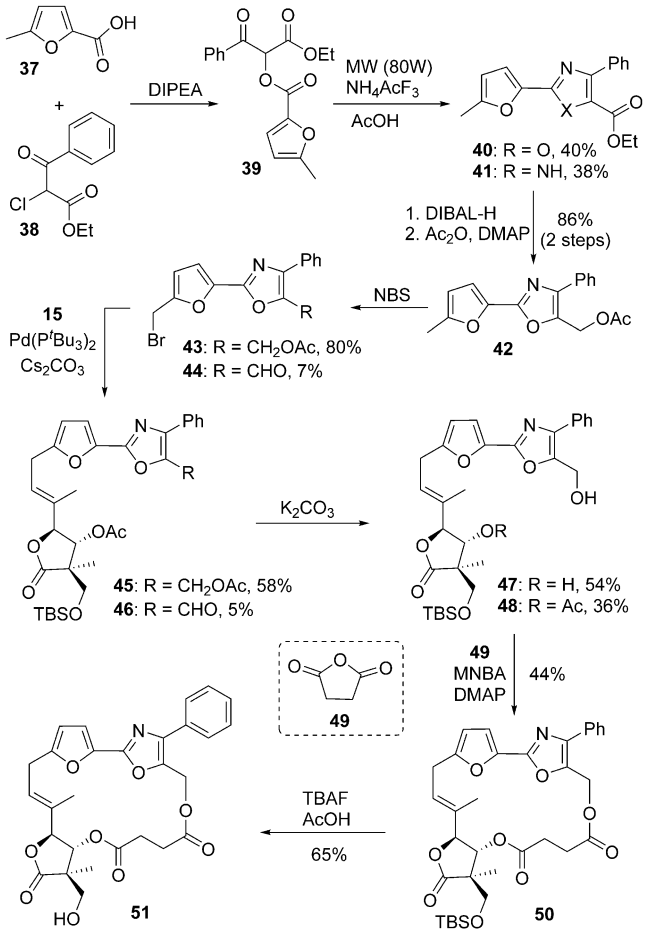

**Scheme 1.** Concise three-step synthesis of simplified Eastern building blocks **12** and **13** and completion of the synthesis of leupylogs **3** and **23**.

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 for leupyrrin synthesis[5] involving $Pd(PtBu_3)_2$, $Cs_2CO_3$ in THF/$H_2O$ 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 amounts were realized with $Pd(PPh_3)_4$ in presence of $K_3PO_4$ 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 ($K_2CO_3$) 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, respectively. Finally, removal of the remaining primary TBS-group proceeded smoothly by utilizing TBAF in THF at 0 °C to obtain leupylogs **4** in 96% and **36** in 61% yield, which proved to be more reliable as compared to TASF.

Inspired by a certain remaining activity of **4** in combination with a high biological tolerance of the diester fragment observed for natural leuyprrins, more truncated leupylog **51** (Scheme 3) was targeted, which contains an oxazol instead of




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




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

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[18] gave the desired oxazole **40** together with imidazole **41**, which could be removed by chromatography. After reduction of the 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 aldehyde stage (**46**, see Supporting Information).

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 $K_2CO_3$ 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 min) and to stop the reaction before full conversion by addition of saturated $NH_4Cl$. This strategy yielded mainly the 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**.

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 Supporting Information).

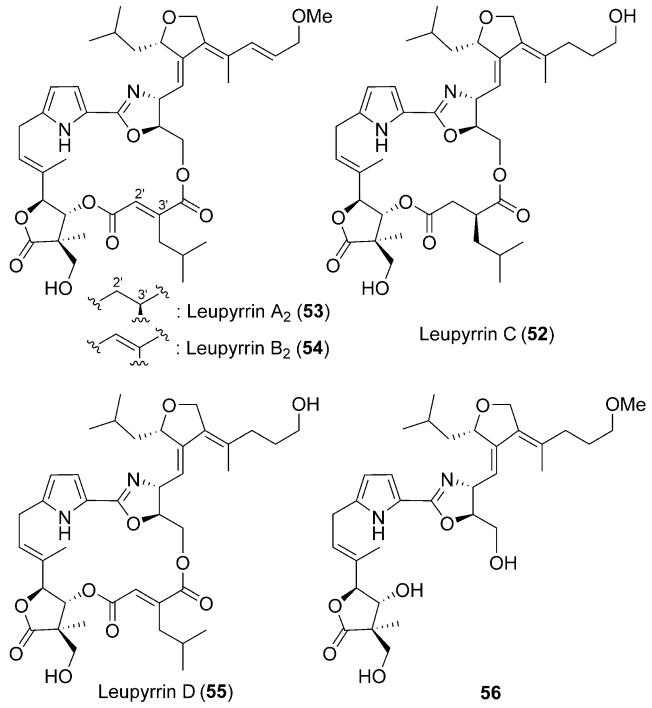



**Figure 2.** Overview of natural leupyrrins **52**–**55** and triol **56**.

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

| | MIC [μg mL$^{-1}$] | | | | | | |
|---|---|---|---|---|---|---|---|
| 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 | 0.94 | 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 | 0.31 | 0.47 | 0.31 | 0.24 | >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].

As outlined in Table 1, all natural leupyrrins show similarly highly efficacious fungal cell growth inhibitory effects in the low μg mL$^{-1}$ 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 Gram-positive 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.

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

**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$^{-1}$] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Compound | **1** | **2** | **3** | **23** | **4** | **36** | **51** | AmB |
| *Rhodotorula glutinis* | 0.29 | 0.14 | 0.23 | 0.21 | 10 | >16 | >16 | 2.45 |
| *Candida albicans* | >16 | >16 | >16 | >16 | >16 | >16 | >16 | 0.33 |
| Mouse fibroblasts (L929) | 0.95 | 2.09 | 9.82 | 5.08 | >40 | >40 | 5.69 | n.d. |

leupylogs **3** and **23** displayed potent $IC_{50}$ 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.

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 μg mL$^{-1}$ and <0.125 μg mL$^{-1}$, respectively). The growth of *Zygosaccharomyces bailii* was impaired, but not completely inhibited at concentrations up to 16 μg mL$^{-1}$. As a control for unspecific toxicity, the yeast *Candida glabrata* that is resistant against natural leupyrrins, was also resistant to **23** (see Supporting Information). While the insensitive organisms are all capable of fermenting glucose, members of the genus *Rhodotorula* possess solely 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 $IC_{50}$ 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 deriv-

atives, 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 $A_1$). 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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## Conflict of interest

The authors declare no conflict of interest.

**Keywords:** antifungal agents · Leupylogs · natural products · SAR studies · total synthesis

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