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Novel Antifungal β-Amino Acids: Synthesis and Activity Against *Candida albicans*

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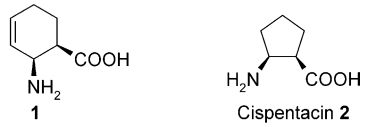
Abstract—A series of novel β-amino acids has been synthesized and tested for their in vitro antifungal activity against *Candida albicans*. A steep SAR was observed. β-Amino acid **21** (BAY 10-8888/PLD-118) revealed the most favourable activity–tolerability profile and was selected for clinical studies as a novel antifungal for the oral treatment of yeast infections.

Introduction

Major increases in the incidence of systemic fungal infections caused by the yeast *Candida albicans* have been observed during the last two decades, particularly in immunocompromised patients.[1] A critical need exists for new antifungal agents to treat these life-threatening infections.[2]

The 2-aminocyclohexenecarboxylic acid **1**, originally designed as pyridoxal phosphate suicide inhibitor, turned out to also have activity against *C. albicans*[3] (Scheme 1). However, in toxicological studies **1** showed a less favourable profile. Along with the reported antifungal activity of the natural β-amino acid cispentacin **2**[4] this prompted us to initiate a derivatization program to identify cyclic β-amino acids with superior oral efficacy and tolerability.



Scheme 1. Antifungal β-amino acids.

Here we report the synthesis of a variety of representative cyclic β-amino acids (**3**–**51**, Table 1) and their in vitro antifungal activity against *C. albicans*.

Chemistry

Several strategies were employed to synthesize the cyclic β-amino acids listed in Table 1.[5,6]

The synthesis of example **6** was accomplished as described in Scheme 2 starting from dihydropyrane **52**, which was derived from unnatural L-glucose.[7]

Example **21** was prepared in a straightforward manner as depicted in Scheme 3.[8] In the key step a highly enantioselective, quinine-mediated alcoholysis of the *meso*-anhydride **60** provided cinnamyl ester **61** (84% yield) with ee ≥ 97%. Subsequent Curtius rearrangement and Pd-catalyzed removal of the cinnamyl protecting groups afforded **21** with ee ≥ 99.5%. This process was successfully used to produce 5 kg of β-amino acid **21**. The absolute configuration of **21** was assigned by X-ray crystallography. Cispentacin **2** as well as examples **7** and **25** were prepared in an analogous fashion.[8]

The *trans*-diastereomer **26** was obtained via isomerization of the protected β-amino acid ester obtained from the Curtius rearrangement of **61** using DBU in refluxing

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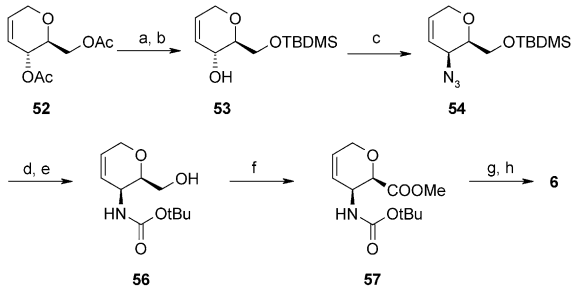
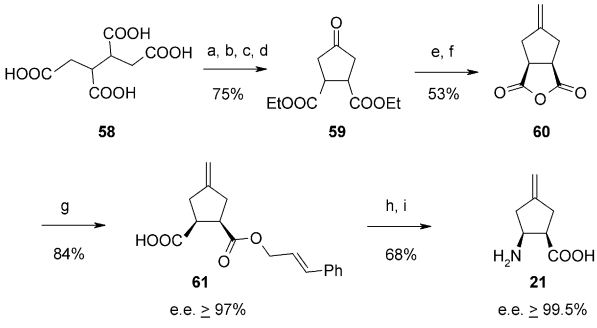
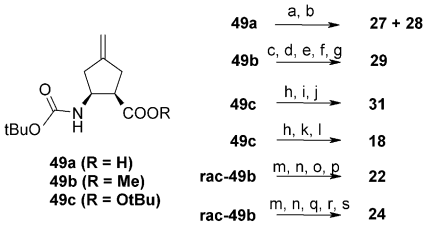


Scheme 2. (a) NaOMe, MeOH, 98%; (b) TBDMSCl, NEt_3, DMAP, CH_2Cl_2, 84%; (c) HN_3, DEAD, PPh_3, THF, 64%; (d) PPh_3, THF, H_2O, then $(Boc)_2O$, NEt_3, CH_2Cl_2, 99%; (e) TBAF, THF, 78%; (f) cat. $RuCl_3$, $K_2S_2O_8$, KOH, H_2O, CH_2Cl_2, then, CH_2N_2, Et_2O, 42%; (g) 4 N HCl, dioxane, 85%; (f) 3 N HCl, reflux, 100%.



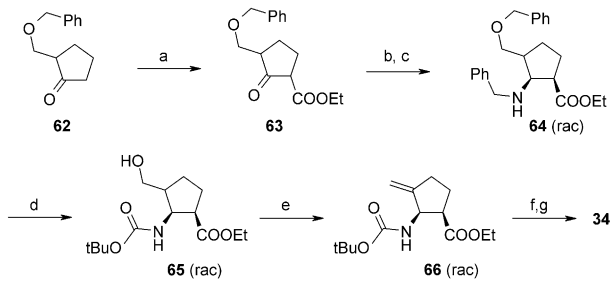
Scheme 3. (a) EtOH, H_2SO_4; (b) NaOMe MeOH; (c) HCl, H_2O; (d) EtOH, H_2SO_4, 75%; (e) $Ph_3PMe^+Br^-$, KOtBu, THF, then KOH, THF, H_2O, 71%; (f) $(EtCO)_2O$, 135 °C, 75%; (g) 1.0 equiv quinine, 1.5 equiv (2E)-3-phenyl-2-propane-1-ol, toluene, −15 °C, 4 h, 84%; (h) $(PhO)_2PON_3$, NEt_3, toluene, 90 °C, then 3-phenyl-2-propane-1-ol, toluene, reflux, 80%; (i) 0.05 mol% $Pd(OAc)_2$, PPh_3, morpholine, EtOH, 85%.



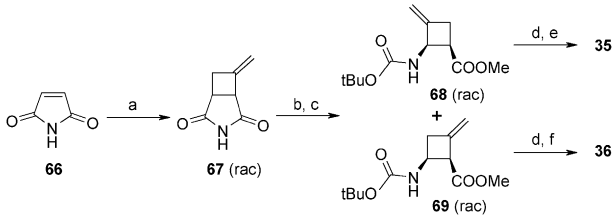
Scheme 4. (a) TMSCl, NaI, CH_3CN, then, FMOC-OSu, Na_2CO_3, H_2O, dioxane; chromat. separation of isomers, 15 and 22%; (b) piperidine, 65%; (c) NMO, SeO_2, CH_2Cl_2, MeOH, chromat. separation of isomers, 28%; (d) H_2, Pd/C, EtOH, 93%; (e) MsCl, NEt_3, CH_2Cl_2, then DBU, THF, 93%; (f) LiOH, THF, H_2O, 91%; (g) 4 N HCl, dioxane, 100%; (h) $Br_3CCOONa$, $BnNEt_3Cl$, $CHCl_3$, 74%; (i) MeLi, Et_2O, −10 °C, 26%; (j) TBDMS-OTf, 2,6-lutidine, CH_2Cl_2, 49%; (k) Bu_3SnH, hexane, 46%; (l) 4 N HCl, dioxane, 100%; (m) O_3, Me_2S, MeOH, 93%; (n) $NaBH_4$, MeOH, 80%; (o) 4 N HCl, dioxane, 97%; (p) HCl, H_2O, 80 °C, 83%; (q) HN_3, DEAD, PPh_3, THF, 81%; (r) LiOH, H_2O, THF, 96%; (s) H_2, Pd/C, 0.1 N HCl, EtOH, then, 4 N HCl, dioxane, 78%.

toluene. Several analogues were synthesized starting from the protected β-amino acid **49** as key intermediate (Scheme 4).

Another series of analogues was synthesized via reductive amination of β-keto esters following the general



Scheme 5. (a) LDA, THF, −78 °C, then EtO_2C-CN, DMPU, 61%; (b) $PhCH_2NH_2$, cat. pTsOH, CH_2Cl_2, 54%; (c) 60 bar H_2, Pt/C, EtOH, 35 °C, 76%; (d) 3 bar H_2, Pd/C, 0.1 N HCl, EtOH, H_2O, then $(Boc)_2O$, NEt_3, CH_2Cl_2, 98%; (e) 2-nitrophenyl selenocyanate, $P(nBu)_3$, THF, then H_2O_2, 90%; (f) LiOH, H_2O, THF, 98%; (g) TBDMS-OTf, 2,6-lutidine, CH_2Cl_2, 52%.



Scheme 6. (a) Allene, CH_2Cl_2, hv, −70 °C, 43%; (b) KOCl, KOH, H_2O, then, $(Boc)_2O$, Na_2CO_3, dioxane; (c) DCC, DMAP, MeOH, CH_2Cl_2, 34% (**68**) and 8% (**69**); (d) LiOH, H_2O, THF; (e) 4 N HCl, dioxane, 68%; (f) TBDMS-OTf, 2,6-lutidine, CH_2Cl_2, 23%.

procedure described in Scheme 5, exemplified by the synthesis of compound **34** starting from **62**.[9]

Further analogues were prepared via Hofmann degradation with hypochlorite as exemplified in Scheme 6 for the synthesis of analogues **35** and **36**.

The trifluoromethyl substituted derivative **42** was prepared from cispentacin **2** by treatment with SF_4/HF at 110 °C.[10] Compounds **43** and **44** were obtained starting from diethyl 2-oxocyclopentanephosphonate[11] and 2-oxocyclopentanesulfonic acid,[12] respectively, via hydrogenation of the corresponding oximes in the presence of PtO_2 (cat.)/Ac_2O.

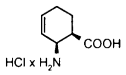
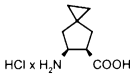
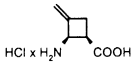
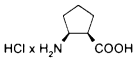
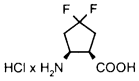
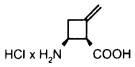
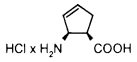
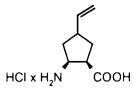
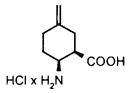
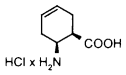
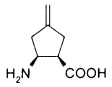
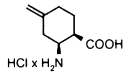
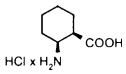
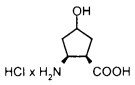
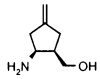
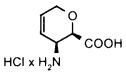
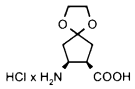
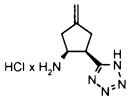
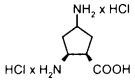
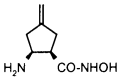
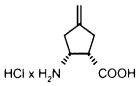
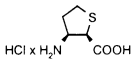
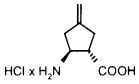
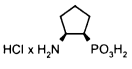
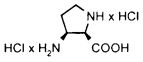
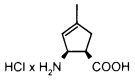
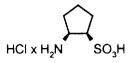
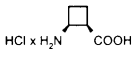
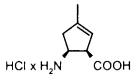
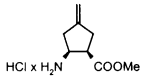
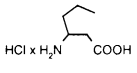
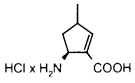
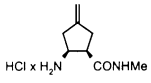
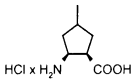
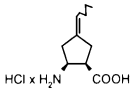
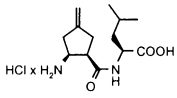
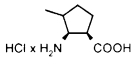
Other examples were synthesized as described or in analogy to known methods,[5, 13–15] for example, via cycloaddition of chlorosulfonyl isocyanate to the corresponding alkenes.[5]

Results and Discussion

All compounds were evaluated for their inhibitory activity against *C. albicans* (Table 1).[16]

2-Aminocyclohexenecarboxylic acid **1** and cispentacin **2** showed potent antifungal activity in this assay (IC_{50} 0.03 and 0.13 mg/L, respectively).

Table 1. In vitro activity of β-amino acids against *Candida albicans*

Compd	Structure	IC_{50} (mg/L)	Compd	Structure	IC_{50} (mg/L)	Compd	Structure	IC_{50} (mg/L)
1		0.03	**18**		32	**35**[a]		>256
2		0.13	**19**[a]		128	**36**[a]		>256
3[a]		0.5	**20**[a] (3:1 m.d.)[b]		128	**37**[a]		>256
4[a]		128	**21**		0.13	**38**[a]		16
5[a]		128	**22**[a] (3:1 m.d.)[b]		128	**39**		>256
6		64	**23**[a]		32	**40**[a]		128
7		64	**24**[a] (5:1 m.d.)[b,c]		64	**41**[a]		32
8[a]		32	**25**		128	**42**[a]		1
9[a]		>256	**26**		128	**43**[a]		>256
10[a]		>256	**27**		8	**44**[a]		>256
11[a]		128	**28**		32	**45**		2
12[a]		>256	**29** (3:1 m.d.)[b,c]		16	**46**[a]		32
13 (5:1 m.d.)[b,c]		4	**30**[a] (2:1 m.d.)[b,c]		128	**47**		16
14[b] (4:1 m.d.)[c]		128	**31**		128	**48**		2
15[a]		diast. A[c] 8 diast. B[c] 16	**32**[a] (single diast.)[c]		64	**49**		16
16[a]		64	**33**[a] (2:1 m.d.)[b,c]		128	**50**[a]		128
17[a]		256	**34**[a]		32	**51**		32

[a]Racemic.
[b]m.d., mixture of diastereomers.
[c]Configuration not known.

A very limited study on the structure–activity-relationship (SAR) of cispentacin **2** as previously described[13] indicated strict structural requirements for antifungal activity. The same trend could be observed in this more extensive study. Whereas dehydro-cispentacin **3** showed only slightly lower potency (IC_{50} 0.5 mg/L), transposition or hydrogenation of the double bond in **1** resulted in a significant loss of activity (examples **4**, **5**). Likewise, insertion of heteroatoms (examples **6**–**10**), reduction of ring size (**11**) or methyl substitution (**13**–**17**) had a negative impact on antifungal activity. A variety of open-chain analogues, as exemplified by compound **12**, were inactive.

We also investigated the SAR at position 4 of the cyclopentane ring in more detail. Among these derivatives (**18**–**24**), only the introduction of an *exo*-methylene group resulted in strong antifungal activity. Compound **21** (IC_{50} 0.13 mg/L) was equipotent to cispentacin **2** and selected for further derivatizations.

The (1*R*, 2*S*)-configuration of **21** turned out to be essential, since stereoisomers **25** and **26** demonstrated only weak antifungal activity. Again, a very steep SAR was observed, resulting in significant loss of potency, when the double bond of **21** was shifted to other positions (**27**–**29**, **34**), small substituents were introduced (**30**–**33**) or the ring size was altered (**35**–**38**). Modifications of the carboxyl (**39**–**47**) and the amino substituent (**48**–**51**) indicated that both groups are crucial for potent in vitro activity against *C. albicans*. Methyl ester **45** and dipeptides such as **48**, however, showed strong in vivo antifungal activity probably due to proteolytic cleavage in plasma to release **21**.

β-Amino acid **21** (BAY 10-8888) exhibits its antifungal activity by a unique dual mode of action.[17] First, it is accumulated about 200-fold in yeast cells by active transport via permeases specific for branched-chain amino acids. Inside the cell **21** inhibits specifically isoleucyl-tRNA synthetase, resulting in inhibition of protein synthesis and cell growth. In contrast, active transport and inhibition of protein synthesis of cispentacin **2** appears to be mediated by the corresponding enzymes specific for proline.[17] Acting as mimetics of small amino acids may explain the observed narrow SAR of antifungal β-amino acids.

Among all so far prepared β-amino acids **21** exhibited the most favourable activity-tolerability profile and was selected for further development. It showed high efficacy in rat and mouse systemic candidiasis models including azole-resistant strains[18] and a favourable pharmacokinetic (almost 100% oral bioavailability in rats, dogs, rabbits; 7 h half-life in man) and safety profile.

In conclusion, an extensive chemical optimization on β-amino acids revealed very steep SAR for antifungal activity and led to the identification of the novel β-amino acid **21**. An efficient asymmetric synthesis could be developed for this compound. BAY 10-8888 **21** is currently being investigated in phase II clinical studies as PLD-118 by Pliva, Croatia for the oral treatment of yeast infections.

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