SYNTHESIS AND ANTIFUNGAL ACTIVITY OF FR109615 ANALOGS

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In our previous paper, we described FR109615, a new antifungal antibiotic, that exhibited excellent *in vitro* and *in vivo* antifungal activity against *Candida albicans*[1,2]. Its structure was determined to be (1*R*,2*S*)-2-aminocyclopentane-1-carboxylic acid (**1**, (−)-*cis*-2-ACPC, Fig. 1)[3,4]. The simplicity and uniqueness of this structure in comparison to known antifungal agents led us to the synthesis of three types of (±)-*cis*-2-ACPC analogs (**2**~**13**, Fig. 2). Their synthesis and antifungal activity is described in this paper.

The β-amino acid analogs **3**~**7** were prepared by the reaction of the corresponding vinylic compounds with chlorosulfonyl isocyanate and hydrolysis

Fig. 1. Structure of FR109615.

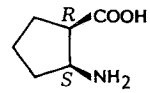

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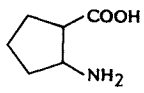
Fig. 2. (±)-*cis*-2-ACPC analogs.



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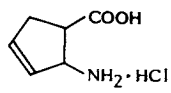

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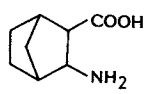

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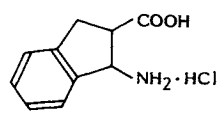

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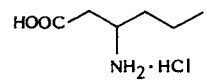

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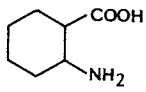

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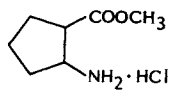

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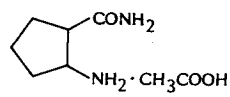
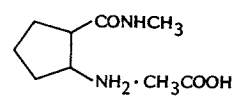


9a



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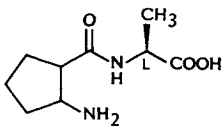
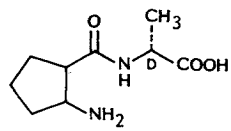


10a



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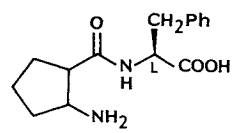
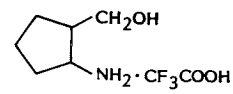


10c



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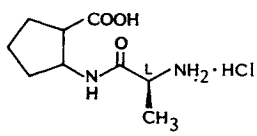
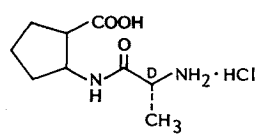


12a



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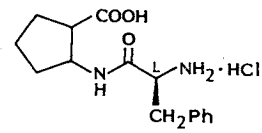
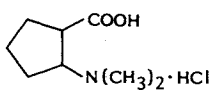


12c



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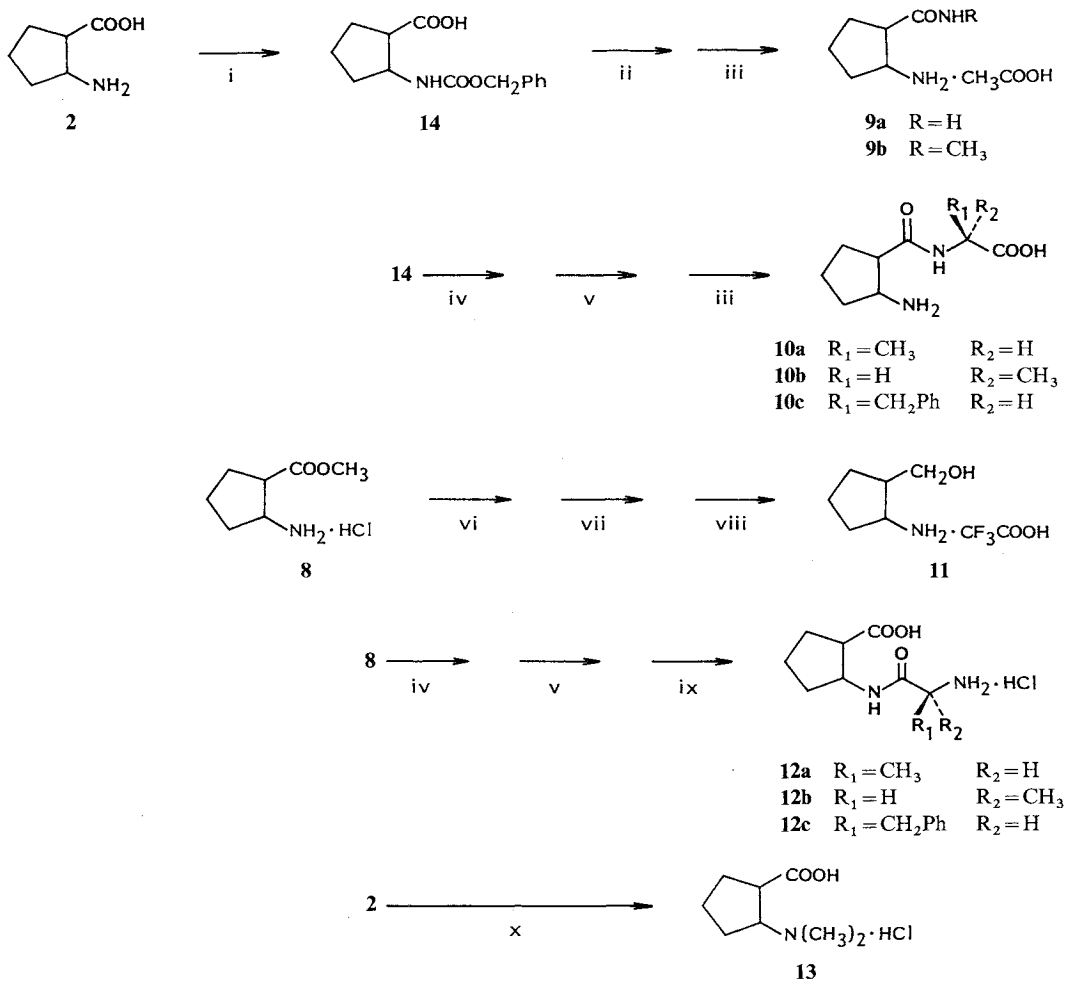
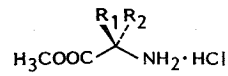
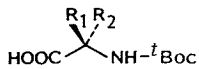
Scheme 1.



(i) $PhCH_2OCOCl$, pH 8.0 ($NaHCO_3$), H_2O, (ii) $ClCOOCH_2CH(CH_3)_2$, Et_3N, RNH_2, THF, (iii) H_2, MeOH-CH_3COOH-H_2O, 5% Pd-C, (iv) WSCD, HOBT,  for preparing **10**,  for preparing **12**, (v) NaOH (1.5 equiv), MeOH-1,4-dioxane-H_2O, (vi) $(^tBoc)_2O$, THF, (vii) $LiAlH_4$, THF, (viii) CF_3COOH, CH_2Cl_2, (ix) 4N HCl in 1,4-dioxane, (x) HCOOH, HCHO.

according to the procedure in the literature[5~8]. Preparation of the derivatives **9**~**13** modified at the carboxyl and amino group are illustrated in Scheme 1. The amino group of (±)-*cis*-2-ACPC (**2**)[3] was protected by the benzyloxycarbonyl (*Z*) group to give **14**. The carboxyl group of the protected compound **14** was activated with isobutyl chlorocarbonate and triethylamine, and treated with the corresponding amines, followed by deprotection of the *Z* group, to give the amide derivatives **9a** and **9b**. The carboxyl group of **14** was activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSCD) and 1-hydroxybenzotriazole (HOBT), and then treated with amines. Hydrolysis and removal of the *Z* group afforded the amino acid derivatives **10**. The amino group of the β-amino ester **8**[3] was protected with di-*tert*-butyl dicarbonate ($(^tBoc)_2O$), followed by reduction and deprotection to give the amino alcohol **11**. The amino ester **8** was acylated with the Boc-amino acids, followed by hydrolysis

Table 1. Antifungal activity of (±)-*cis*-2-ACPC analogs (**1**~**13**) against *Candida albicans* and *Candida krusei*.

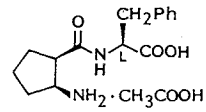
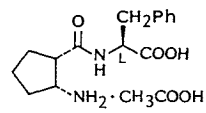
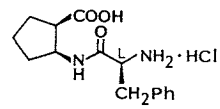
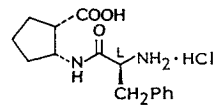
Compound No.	MIC (μg/ml)		
	C. albicans Yu-1200	*C. albicans* FP-579	*C. krusei* FP-585
FR109615 ((−)-*cis*-2-ACPC (**1**))	3.13	3.13	6.25
2 ((±)-*cis*-2-ACPC)	6.25	25	12.5
3	25	100	50
4	>100	>100	>100
5	>100	>100	>100
6	>100	>100	>100
7	>100	>100	>100
8	100	100	>100
9a	>100	>100	>100
9b	>100	>100	>100
10a	1.56	3.13	>100
10b	100	>100	>100
10c	25	12.5	>100
11	>100	>100	>100
12a	12.5	12.5	>100
12b	>100	>100	>100
12c	6.25	6.25	>100
13	>100	>100	>100

EAGLE's MEM agar (Nissui), 10^5 cfu/ml. Streak method, 30°C, 24 hours.

and removal of the Boc group to give the amino acid derivatives **12**. The dimethylamino derivative **13** was prepared by refluxing **2** in a solution of 90% formic acid and formalin.

In vitro antifungal activity of the synthesized (±)-*cis*-2-ACPC analogs against *C. albicans* and *Candida krusei* are summarized in Table 1. Among the β-amino acid analogs **3**~**7**, only the cyclopentene derivative **3** showed reasonable activity. In contrast the open-chain compound **6**, the condensed-ring compounds **4**, **5** and the cyclohexane derivative **7** had no activity. It was found that the monocyclic cyclopentane was essential for potent antifungal activity. Among the derivatives modified at the carboxyl and amino group, the ester **8**, the simple amides **9a**, **9b** and the primary alcohol **11** were inactive. The dimethylamino compound **13** was also inactive. From these results it would seem that the carboxyl and primary amino groups were necessary for potent antifungal activity. However several dipeptides including this unusual amino acid also exhibited potent anticandidal activity. The stereochemistry of the amino acid of these dipeptides was critical to activity, and the difference between the L- and the D-amino acid derivatives was remarkable. Thus, the L-amino acid derivatives **10a**, **10c**, **12a** and **12c** exhibited potent activity against *C. albicans* and no activity against *C. krusei*, whereas the D-amino acid derivatives **10b** and **12b** were almost inactive.

Table 2. Antifungal activity of (+)- and (−)-*cis*-2-ACPC derivatives (**15**~**18**) against *Candida albicans*.

Compound No.	MIC (μg/ml)	
	C. albicans Yu-1200	*C. albicans* FP-579
FR109615 (**1**)	3.13	6.25
 **15**	6.25	25
 **16**	>100	>100
 **17**	1.56	3.13
 **18**	>100	>100

EAGLE's MEM agar (Nissui), 10^5 cfu/ml. Streak method, 30°C, 24 hours.

Furthermore the optical isomers **15**, **16**, **17**, and **18** of the L-amino acid derivatives **10c** and **12c** were synthesized with the same method as the synthetic method of **10c** and **12c** by using the (−)- and the (+)-*cis*-2-ACPC[3] as the starting materials, and their antifungal activity is tested (Table 2). As the result, the (−)-*cis*-2-ACPC derivatives **15** and **17** exhibited potent activity against *C. albicans*, whereas the (+)-*cis*-2-ACPC derivatives **16** and **18** were almost inactive.

In summary, it seems that there are strict structural requirements for antifungal activity, among these series of (1*R*,2*S*)-2-aminocyclopentane-1-carboxylic acid analogs and derivatives.

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