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SYNTHESIS AND EVALUATION OF BACKBONE/AMIDE-MODIFIED ANALOGS OF LEUALACIN

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Abstract:. Leualacin (**1**), a cyclic depsi-pentapeptide, and its backbone/amide-modified analogs **2-4** were synthesized. Amide analogue **3** exhibited stronger vasodilatory effects. It also strongly inhibited collagen- and arachidonic acid (AA)-induced platelet aggregations with IC_{50}s of 0.6 μM and 2.0 μM, respectively.

On the pursuit of peptides as potentially biological candidates, cyclic structures reduce peptide conformational freedom and often result in high receptor binding affinity by reducing unfavorable entropic effects.[1] By these reasons, the cyclic peptides often make promising lead compounds for drug discovery. Leualacin (**1**), a cyclic depsi-pentapeptide isolated from the fungus *Hapsidospora irregularis*,[2] contains unusually two esteric bridges on its skeleton. Preliminary studies revealed that leualacin acts specifically on the dihydropyridine-sensitive Ca^{2+} channel with potency similar to the known calcium channel blockers such as diltiazem and nitrendipine.[3] It is expected that Ca^{2+} channel blockers with distinct structures/properties would be valuable for new pharmacological applications to the cardiovascular diseases such as variant angina and thrombosis. In this report, we specifically modified the least hindered esteric bridge and amide moiety of leualacin and observed their activities on vasodilatory and antiplatelet effects.

Chemical synthesis. The strategy for the synthesis of the cyclic peptides is usually using fragment couplings followed by macrocyclization between the least hindered residues.[4] Thus, the synthesis of leualacin and its backbone/amide analogs **2-4** utilized 2 + 3 fragment couplings followed by intramolecular cyclization between the least sterically demanding β-alanyl and phenylalanyl residues (Scheme **2**).[5] The synthesis of required (depsi)-dipeptides fragments **10a-d** and depsitripeptide **17** were starting from D-leucine and L-leucine, which were each transferred with retention into R-leucic acid (**6**) and S-leucic acid (**11**)[6] (Scheme **1**). Carboxyl protections of **6** and **11** as diphenylmethyl esters were accomplished with diphenyldiazomethane[7] to afford benzhydryl esters **7** and **12**. Boc-β-alanine (**5a**) and Boc-*N*-methyl-*β*-alanine (**5b**)

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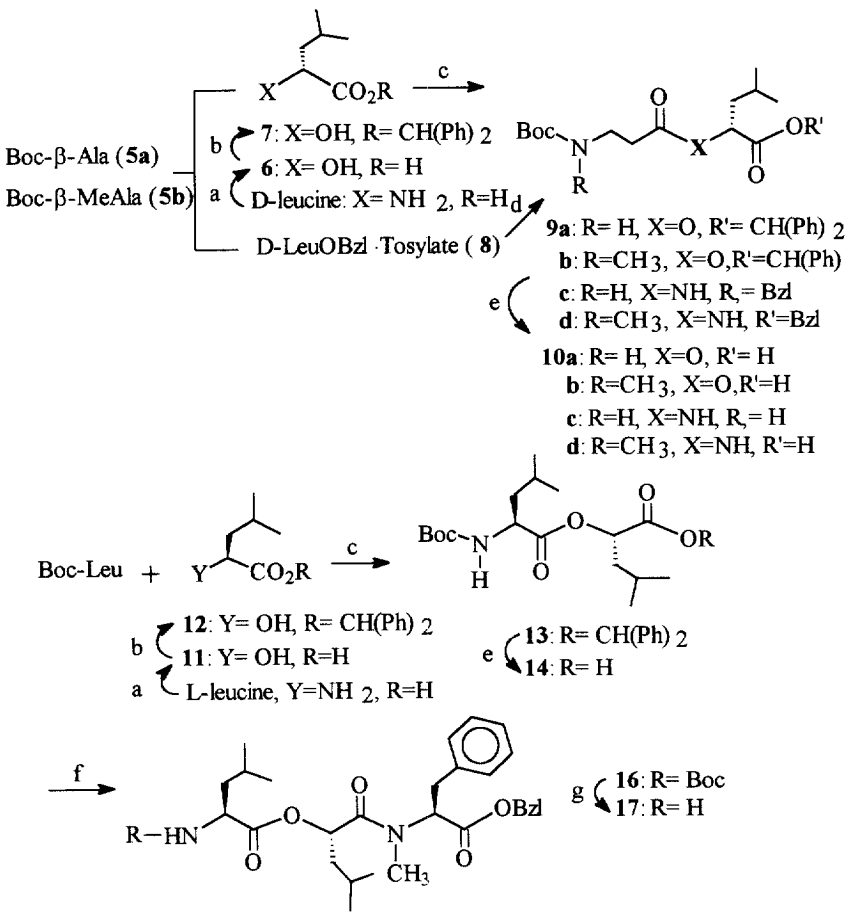
were each coupled with hydroxyl ester **7** using DMAP to afford depsipeptides **9a,b**. Similarly, depsidipeptide **13** was readily obtained by coupling Boc-leucine with alcohol **12**. Further, **5a,b** was each condensed with benzyl D-leucinate tosylate salt (**8**) to give dipeptides **9c,d**.

Scheme 1. Synthesis of (Depsi)-peptides 10a-d and 17



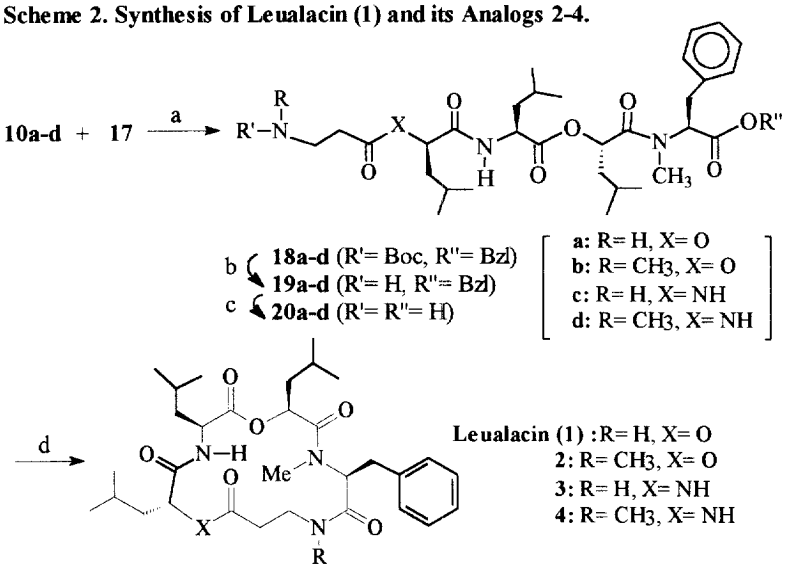
a. $NaNO_2$, H_2SO_4, H_2O, 51% b. $Ph_2C{=}N{-}NH_2$, $PhI(OAc)_2$, I_2, -10 °C, 70%
c. EDCI, DMAP, TEA, 74-89% d. EDCI, HOBt, 50-65% e. H_2, 10% Pd-C, MeOH, EtOAc, 94 - 98% f. MePheOBzl ·Tosylate (**15**), BOP-Cl, NMM, 84%
g. TFA, -10 °C, 95%.

Catalytic hydrogenolysis of benzhydryl ester **9a**, **b**, and **13** and benzyl esters of **9c,d** readily gave acids **10a-d** and **14**. BOP-Cl-activated coupling of **14** and H-

MePheOBzl tosylate (**15**), prepared from TsOH-catalyzed benzylation with concomitant removal of *N*-protected Boc group of Boc-MePhe-OH,[8] gave the depsipeptide **16**. Acid-catalyzed removal of the Boc-group of **16** provided the only depsi-tripeptide **17** with an overall 80% yield.

With (depsi)-peptides **10a-d** and **17** in hands, BOP-Cl-mediated 2 + 3 fragment coupling was found to proceed with a variation in yields (Scheme **2**). The lowest yield (51%) of pentapeptide **18c** was obtained in the coupling of **10c** (R= H, X= NH) with **17**, whereas coupling between **10d** (R= CH_3, X= NH) and **17** gave the best result (97%). In other run, high yield (89%) of **18a** was obtained by using PyBroP[9] as the coupling reagent. Further removal of N- and C-terminal protecting groups of (depsi)-pentapeptide analogs **18a-d** was accomplished in a similar manner to those conditions used in the preparation of the fragments and proceeded with high efficiency to provide free (depsi)-pentapeptides **20a-d**, which were readily used without further purification.

Scheme 2. Synthesis of Leualacin (1) and its Analogs 2-4.



a. BOP-Cl, DIEA, 0 °C , 51-97% b. TFA, -10 °C, 83-96% c. H_2, Pd-C, MeOH, EtOAc, 92-96% d. DPPA, DMAP, 2 d, 30-52%.

The remaining macrocyclization of these linear (depsi)-pentapeptides was found to be a more problematic step in the overall synthesis of leualacin analogs. Initial attempt at cyclization of **20a** utilized diphenylphosphoryl azide (DPPA)[10] as an activating agent in the $NaHCO_3$ / DMF conditions. Surprisingly no desired product was obtained. By previous experiences on the synthesis of cyclopeptides,[11] we next

tried to use propylphosphonic anhydride (PPA) and Castro's BOP reagent as activating agents. Still these reagents did not drive cyclization at all. Finally, under DPPA / DMAP and high dilution conditions (in 1 x 10^{-4} molar of CH_2Cl_2) for two days, linear pentapeptides **20a-d** were cyclocondensed to produce leualacin (**1**) and its analogs **2-4** in 30-52% yields.[12]

Biological results and Discussion: Leualacin and its analogs **2-3** were evaluated in the isolated aorta from WKY rats for Ca^{2+}-antagonistic effects according to the assay method as previously described. [13] Data from vasodilatory effects were summarized in Table **I**. The relaxation induced by these compounds in aortic rings contracted by 60 mM K^+ were mostly in a concentration-dependent manner. Amide analogue **3** exhibited 100% relaxation at 10^{-6} M level in isolated rat aortic rings, whereas *N*-methyl analogue **2** showed no improvement compared to leualacin (26% relaxation). The stronger relaxation of **3** may be explained by the more stable amide-bond and still mimicking to the conformation of the backbone of leualacin.

Table 1. Vasodilatory effect of leualacin and its analogs on aortic arch of rat

conc. (M)	vasodilatory rate (%)[a]		
	leualacin(**1**)	**2**	**3**
10^{-4}	66.5±31.4	78.0±22.6	---[b]
10^{-5}	64.4±7.1	80.8±4.0	---
10^{-6}	26.8±10.1	25.1±1.0	100.0±5.5
10^{-7}	---	32.5±2.7	35.0±6.6
10^{-8}	---	11.5±6.5	29.3±2.4

[a] Values in rate (%)± standard error (n=3)

[b] --- no data

With regard to the potentially antithrombotic activity, leualacin and analogs **2-4** were also evaluated with inhibitory effects on collagen-, arachidonic acid (AA)-, and U46619, a thromboxane A_2 receptor agonist, -induced platelet aggregations as previously described.[14] As seen in the Table **2**, 50% of inhibition of collagen (10 μg/mL)-induced aggregation of platelets was achieved at the ranges of concentration of 50±4 μM by leualacin and 10±2 μM by **3**. Platelet aggregation induced by AA and U46619 was similarly inhibited by leualacin and **3** with higher IC_{50} values (150± 8 μM vs 30 ~ 40±4 μM). These data indicated that **3** displayed about 5 times more potent on the inhibition of platelet aggregations and antagonistic effects toward the thromboxan A_2 receptor than did leualacin. Analogs **2** and **4** showed no significant inhibitions on these experiments with concentrations higher than 150 μM. Further

evaluation for leualacin and **3** on the inhibition of the formation of thromboxane B_2 (TXB_2) induced by collagen and AA was accomplished and shown in the Table **3**. At the same concentration level (100 μM), **3** also displayed stronger effects than leualacin on the inhibition of TXB_2 formation induced by both collagen (25.6±2.4 μM vs 39.4±3.5 μM) and AA (30.7±9.2 μM vs 55.4±18.8 μM).

Table 2. Inhibitory effect of leualacin and its analogs on platelet aggregation

IC_{50} (μM) / compound	stimulant		
	collagen (100 ug/mL)	arachidonic acid (100 μM)	U46619 (1 μM)
leualacin (**1**)	50±4	150±8	150±7
2	>150	>200	>200
3	10±2	30±4	40±3
4	>150	>200	>200

(n = 5-7)

Table 3. Inhibitory effect of leualacin and analogue 3 on TXB_2 formation by platelets in response to collagen and arachidonic acid

compound (100 μM)	amounts of TXB_2 formed (ng/mL)	
	collagen (100 μg/mL)	arachidonic acid (100 μM)
control	65.3±4.0	126.1±18.8
leualacin (**1**)	39.4±3.5*	55.4±8.6*
3	25.6±2.4*	30.7±9.2*

The stimulant was added to the suspension of test drug or DMSO (0.5%, control) and platelets, which has been incubated for 1 min at 37 °C. The suspension was treated with EDTA (2 mM) and followed by indomethacin 6 min later. After centrifugation, the amount of TXB_2 in the supernatent portion was detected by EIA as shown (n= 4).
* $P < 0.05$ relative to control.

In brief, amide analogue **3** demonstrated that both vasodilatory and antiplatelet activities of leualacin could be improved classically by modification of the least hindered esteric bridge with a stable amide. This finding proved that the esteric bridge between D-leucic and β-alanyl residues at leualacin is not a requisite for these activities. These results also represent an important feature that leualacin could be a potential lead for developing new types of cardiovascular agents.

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12. All compounds were characterized by ^{1}H NMR, Mass, and HR-FABMS. Spectral data of representative compound **3**: R_f= 0.40 (70% EtOAc/hexane); mp 128-130 ℃; [α] +297° (c 0.06, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.41 (d, 1H, J = 9.1 Hz, NH), 7.30-7.20 (m, 5H, Aromatic's H), 7.07 (d, 1H, J = 9.8 Hz, NH), 6.53 (d, 1H, J = 9.8 Hz, NH), 5.63 (d, 1H, J = 8.5 Hz, α-CH), 4.72 (dd, J = 11.5, 6.3 Hz, 2H, 2α-CH), 4.47 (m, 2H, 2α-CH), 4.04 (m, 1H, β-CH), 3.40 (m, 2H, 2β-CH), 2.95 (m, 1H, β-CH), 2.84 (s, 3H, N-CH_3), 2.52 (d, 1H, J = 17.3 Hz, α-CH), 2.20 (m, 1H, α-CH), 1.79 (m, 2H, 2β-CH), 1.75, 1.65 (2m, 2H, 2γ-CH), 1.54 (m, 1H, β-CH), 1.46 (m, 1H, γ-CH), 1.42 (m, 1H, β-CH), 1.20 (m, 1H, β-CH), 0.98-0.80 (m, 12H, 4CH_3), 0.67, 0.54 (2d, 6H, J = 6.5 Hz, 2CH_3); FABMS (Gly as matrix): m/z $[M+H]^+$ 573.0; HR-FABMS exact mass calcd for $C_{31}H_{49}N_4O_6$ $[M+H]^+$ 573.3652, found 573.3703.
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