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Antibradykinin Effects of the Non-peptide Antagonists of Mixture Libraries Prepared by Solution-phase Combinatorial Synthesis

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Abstract – The solution-phase combinatorial synthesis of iminodiacetic acid triamide libraries linked to 1-(4-chlorobenzhydryl)piperazine has been reported. Ten mixture libraries, each containing 5 components, were synthesized in 4 steps from *N*-BOC-iminodiacetic acid anhydride. Antibradykinin effects of the mixture and individual libraries were compared using guinea-pig ileum smooth muscle. The changes in the inhibition were also observed by testing the combination of two different compounds from the same library. We found out the correlation between the inhibition of mixtures and that of individual libraries. It is possible to choose the mixtures with relatively high inhibitory effects to find out the most effective individual compound for further synthesis.

Keywords □ solution-phase combinatorial synthesis, bradykinin receptor antagonists, non-peptide, guinea-pig ileum smooth muscle contraction, chlorobenzhydryl piperazine

INTRODUCTION

Given its ability to rapidly and efficiently produce large number of diverse compounds in a cost-effective manner in conjunction with high-throughput screening of the increasing number of molecular targets, combinatorial synthesis has emerged as a powerful tool for the acceleration of the drug discovery process. The implications of technology are apparent arising from the production of smaller targeted libraries for optimization around a promising lead candidate. The solution phase, parallel synthesis of chemical libraries allows the preparation of multi-milligram quantities of each individual member (Tarby *et al.*, 1996).

In each step of the sequence, the reactants, unreacted starting material, reagents and their byproducts were removed by simple liquid/liquid extractions. Its non-limiting scale, expanded and non-limiting repertoire of chemical reactions, direct production of soluble intermediates and final products for assay or for purification, and the avoidance of linking and capping strategies make solution-phase combinatorial synthesis an attractive alternative (Cheng *et al.*, 1996; Boger *et al.*, 1999). We have been reported the solution-phase combinatorial synthesis and pharmacological effect of fifty *N*, *N'*-substituted-*N''*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide derivatives as nonpeptide B_2 antagonists. (Kam *et al.*, 2004 and 2005). The differences in the antibradykinin effects between those mixtures and individual libraries were compared using guinea-pig ileum smooth muscle in this study. We also tested the antibradykinin effects of two differently combined compounds of the same library to observe the possible interaction between those compounds.

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MATERIALS AND METHODS

General procedure for the synthesis of mixture libraries:

$CHCl_3$ (1 mL) and 4 M HCl-dioxane (1 mL) were added to **A1B1-A10B1** (0.169 mmol) in a 4 mL vial and this mixture was allowed to stand for 3 h. The solvent and excess acid were removed by evaporation. The resulting residue was dissolved in 1 mL of DMF and 3.09 mmol of *i*-Pr_2NEt. After dissolution, 0.0103 mmol of a carboxylic acid stock solution (prepared by diluting a mixture of 0.186 mmol of each carboxylic acid **S1-S5** in 9 mL of a mixture DMF/ $CHCl_3$ 8:1) was added, followed by 0.169 mmol of PyBrOP. After stirring for 16 h, the reaction mixture was diluted with EtOAc (90 mL) and washed with 10% aqueous HCl (3×90 mL), saturated aqueous $NaHCO_3$ (2× 90 mL), and saturated aqueous NaCl (90 mL), dried (Na_2SO_4), and concentrated to afford the mixture.

Mixture A1B1C1-5

Yellow oil. 33 mg (35%). FABMS *m/z* 485, 547, 561, 597, 611.

Mixture A2B1C1-5

Redish brown oil. 20 mg (20%). FABMS *m/z* 533, 595, 609, 645, 659.

Mixture A3B1C1-5

Orange oil. 11 mg (10 %). FABMS *m/z* 547, 609, 623, 659, 673.

Mixture A4B1C1-5

Orange oil. 30 mg (25%). FABMS *m/z* 563, 625, 639, 675, 689.

Mixture A5B1C1-5

Yellow oil. 41 mg (34%). FABMS *m/z* 575, 637, 651, 687, 701.

Mixture A6B1C1-5

Orange oil. 26 mg (20%). FABMS *m/z* 623, 685, 699, 735, 749.

Mixture A7B1C1-5

Red brown oil. 26 mg (20%). FABMS *m/z* 636, 698, 712, 748, 762.

Mixture A8B1C1-5

Yellow oil. 35 mg (30 %). FABMS *m/z* 566, 628, 642, 678, 692.

Mixture A9B1C1-5

Light brown solid. 112 mg (82%). FABMS *m/z* 680, 742, 756, 792, 806.

Mixture A10B1C1-5

Yellow oil. 18 mg (1%). FABMS *m/z* 525, 587, 601, 6.37, 651.

Bradykinin-induced contractions of guinea-pig ileum

Bradykinin, captopril, indomethacin, dithiothreitol, atropine and HOE140 were obtained from Sigma (St. Louis, MO, USA). All peptides were dissolved and diluted in a Tyrode solution, and dithiothreitol was dissolved in dimethyl sulfoxide (DMSO) and diluted in Tyrode solution. Captopril, indomethacin, atropine, and all synthesized compounds were dissolved and diluted in DMSO. The composition of the Tyrode solution is as follows (in mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.15, NaH_2PO_4 0.4, $NaHCO_3$ 11.9, glucose 5.6. The final DMSO concentration in the bath was less than 0.01%, and it had no discernible effect on the tissue responsiveness to bradykinin.

Male Hartley guinea-pigs weighing 260-500 g (Jeil, Korea) were fasted overnight and then decapitated. A section of ileum approximately 40 cm in length was removed at a level 2 cm above the ileocecal junction and placed in warm (37°C) Tyrode solution. Strips of intestinal muscle with mucosa, 1.5-2 cm in length, were then mounted in a 50 ml bath containing Tyrode solution (37°C) and aerated with 95% O_2/5% CO_2. Tissue contractions were recorded isometrically on a Grass model 76E polygraph.

After an equilibration period of about 60 min, a stable baseline tone was reached and two or three contractions were obtained in response to bradykinin (0.1 μM), at 20-min intervals, to assay the sensitivity and reproducibility of the contractile response. Only segments producing reproducible responses were used. The last control response was taken as 100% and subsequent results obtained with bradykinin antagonists were expressed as a percentage in respect to this. The segments were incubated with the bradykinin-antagonists (0.1 μM) for 5 min before bradykinin was added. To minimize degradation of bradykinin and to prevent responses due to neuronal activation or prostaglandin production, Tyrode solutions contained 1 mM each of captopril, atropine, dithiothreitol, and indomethacin.

Statistical analysis

All results are expressed as the mean ± SEM (n=3~10), and the sample size, n, represents the number of individual strips of ileum assayed. The experiments were designed such that the sample size would also represent the same number of guinea-pigs. For example, two to four strips from one guinea-pig were used for two to four different tests for an n = 1 sample size, and those from another guinea-pig were also used for the same two to four tests to increase the sample size for a given test to n = 2, and so on.

RESULTS AND DISCUSSIONS

Fifty non-peptide bradykinin B_2 receptor antagonists as a 10 ×1×5 matrix were designed and synthesized by solution-phase

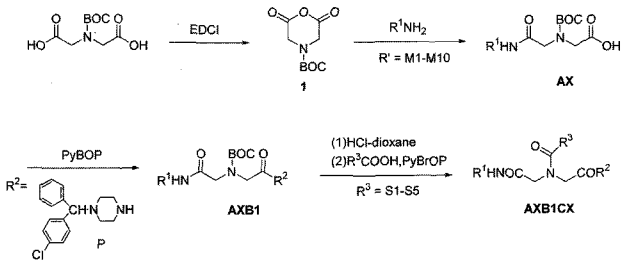

Fig. 1. Solution-phase combinatorial synthesis route using the iminodiacetic acid template.

combinatorial synthesis using the iminodiacetic anhydride template (Figures 1 and 2). All of the mixture libraries showed inhibitory activity on bradykinin-induced contraction at 0.1 μM concentration in the guinea-pig ileum (Tables I). When the % inhibition of each mixture library in Table I was compared with the mean of 5 compounds of the same group in Table II, most of them matched one another very well. However, mixture A4B1C1-5 showed 27.70% inhibition while the mean of five compounds was 16.36% inhibition. Mixtures with high activity, such as A3B1C1-5 and A6B1C1-5, could be chosen for further individual synthesis to find out active compounds. This method saves time and effort by synthesizing 10 compounds and evaluating their biological activity instead of going through all 50 individual compounds.

Among 10 mixture libraries, **A3**, **A4**, **A5** and **A6** series with benzyl or phenyl propyl amines, showed relatively high antibradykinin effects (Table I). Additionally, the individual libraries of those mixtures (**A3**, **A4**, **A5** and **A6**) also showed better inhibitory activities than the others (Table II).

The most potent series (**A3**), compounds **A3B1C1**~**A3B1C4** (Figure 3) was selected for the investigation of their antibradykinin effects by arranging different compounds in pair. The inhibitory effects of these combined compounds were listed in Table III. Antibradykinin tested by each compound

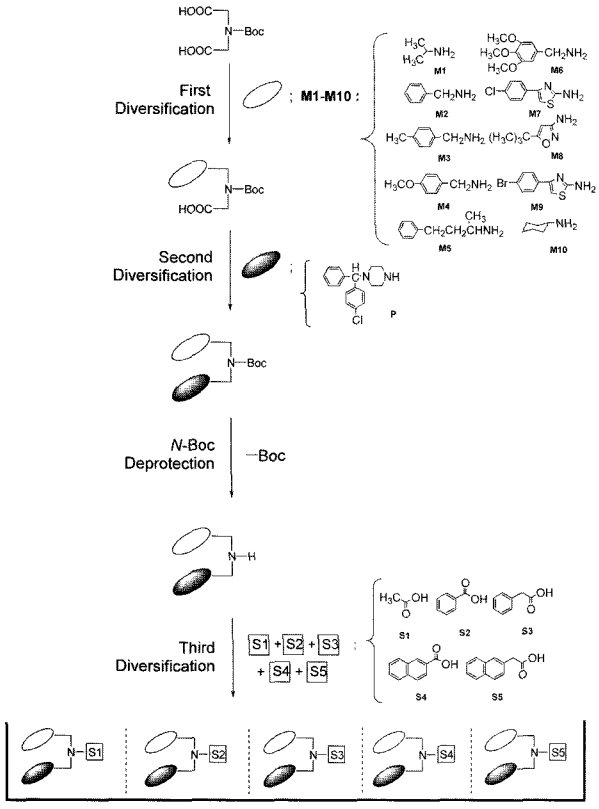

Fig. 2. Synthesis of 10×1×5 library.

Table I. Antibradykinin activity of mixture libraries at 0.1 μM concentration in the guinea-pig ileum

Mixture	% Inhibition
A1B1C2-5	10.59 ± 2.61
A2B1C1-5	22.93 ± 2.67
A3B1C1-5	30.27 ± 5.10
A4B1C1-5	27.70 ± 7.42
A5B1C1-5	25.05 ± 7.25
A6B1C1-5	33.27 ± 6.39
A7B1C1-5	12.18 ± 6.79
A8B1C1-5	21.74 ± 7.82
A9B1C1-5	12.11 ± 3.87
A10B1C1-5	18.04 ± 4.43

Table II. Antibradykinin activity of individual libraries at 0.1 μM concentration in the guinea-pig ileum (Kam et al., 2004)

Sample no.	% Inhibition	Sample no.	%Inhibition
A1B1C1	n.r.*	A2B1C1	12.90±2.04
A1B1C2	7.67±3.48	A2B1C2	19.46±2.22
A1B1C2	13.26±1.72	A2B1C3	18.29±4.78
A1B1C4	23.67±5.78	A2B1C4	19.64±2.98
A1B1C5	7.12±3.44	A2B1C5	16.67±2.95
mean	12.93±3.84	mean	17.39±1.24
A3B1C1	46.32±6.32	A4B1C1	-0.10±2.75
A3B1C2	24.52±2.49	A4B1C2	5.96±3.05
A3B1C3	42.58±0.28	A4B1C3	39.16±5.69
A3B1C4	31.19±6.78	A4B1C4	18.21±3.40
A3B1C5	23.70±3.15	A4B1C5	18.55±2.30
mean	33.66±4.63	mean	16.36±6.73
A5B1C1	19.95±3.58	A6B1C1	20.76±5.39
A5B1C2	27.68±4.72	A6B1C2	18.45±2.47
A5B1C3	24.00±1.98	A6B1C3	32.63±7.77
A5B1C4	25.96±1.72	A6B1C4	22.50±7.04
A5B1C5	33.60±9.60	A6B1C5	21.90±3.89
mean	26.24±2.25	mean	23.25±2.45
A7B1C1	17.96±7.96	A8B1C1	16.57±4.56
A7B1C2	13.38±6.31	A8B1C2	20.82±3.04
A7B1C3	7.30±5.90	A8B1C3	12.90±3.85
A7B1C4	10.86±7.61	A8B1C4	26.02±4.54
A7B1C5	7.78±9.87	A8B1C5	-6.02±7.26
mean	11.46±1.96	mean	14.06±5.48
A9B1C1	12.76±5.84	A10B1C1	0.42±1.38
A9B1C2	8.50±3.91	A10B1C2	13.33±4.82
A9B1C3	9.19±5.13	A10B1C3	8.75°±3.86
A9B1C4	2.16±4.00	A10B1C4	4.71±2.73
A9B1C5	9.90±6.73	A10B1C5	19.15±9.37
mean	8.50±1.74	mean	9.27±3.27
HOE 140	82.22±2.31		

*not reacted.

Table III. Antibradykinin activity of A3 series in combination at 0.1 μm concentration in the guinea-pig ileum.

Sample no.	% Inhibition (tested combined)	% Mean (tested each)
A3B1C1 + A3B1C2	19.50 ± 4.16	35.42 ± 10.9
A3B1C1 + A3B1C3	24.88 ± 7.62	44.45 ± 1.87
A3B1C1 + A3B1C4	15.82 ± 2.98	38.75 ± 7.57
A3B1C2 + A3B1C3	28.86 ± 2.24	33.55 ± 9.03
A3B1C2 + A3B1C4	19.89 ± 2.74	27.85 ± 3.34
A3B1C3 + A3B1C4	30.03 ± 3.27	36.88 ± 5.70

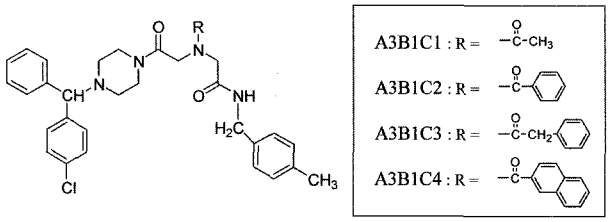

Fig. 3. Chemical structure of compounds **A3B1C1 - A3B1C4**

was better than that of the compounds tested concurrently. Especially, when compounds were combined with **A3B1C1**, the inhibition diminished more than the others. Compounds **A3B1C1** and **A3B1C3**, for example, showed 44.45% of as mean of individual tests, while the antibradykinin effects reduced to 24.88% when the two of them were tested in combination (Table III).

In conclusion, we found out the correlation between the inhibition of mixtures and that of individual libraries. It is possible to choose only mixtures with relatively high inhibitory effects for further synthesis of their individual libraries. This study showed us the possibility that one compound may alter the intensity of antibradykinin effects of another compound given concurrently, and the net result may be enhanced or diminished effects of one or both of the compounds.

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