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Solution-phase combinatorial synthesis of nonpeptide bradykinin antagonists

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Abstract—We describe the solution-phase combinatorial synthesis and pharmacological effect of fifty *N*,*N*′-substituted-*N*″-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide derivatives as nonpeptide B2 antagonists. The synthesized compounds were tested for their antibradykinin activity by utilizing guinea-pig ileum smooth muscle. Most of the compounds showed antagonistic effects on bradykinin induced contraction. *N*-acetyl-*N*′-(4-methylbenzyl)-*N*″-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (**A3B1C1**) showed the 46% inhibition at 100 nM.

1. Introduction

Bradykinin is an autocoid related to acute and chronic pain and inflammation. Two bradykinin receptors B1 and B2 are known and the B2 receptor is constitutively expressed on most cell types, whereas the B1 receptors are induced during inflammatory insults.[1–3] Most of the antagonists for bradykinin B2 receptors reported are bradykinin derived peptides such as **HOE 140**.[4]

The nonpeptide bradykinin antagonists were of interest as novel anti-inflammatory therapeutics. The first nonpeptide antagonist Win 64338 was reported in 1993 by Sterling Winthrop researchers,[5] but it showed low specificity and a species-dependent variable affinity for B2 receptors. Some active compounds such as FR 173657, LF 16-0687, and bradyzide have been reported recently (Fig. 1).[6,7] The derivatives synthesized by Fujisawa showed antagonist activity at the bradykinin B2 receptor and as in FR 173657, they have the structural feature that a dichlorobenzene-linked terminal quinoline stands one side, while the other side varies from basic heterocycles to amidinobenzene. Especially the FR 173657 is a potent, orally available B2 antagonist, and its biological activity has been studied extensively.[8–10] On the other hand, Fournier compound LF 16-0687, which is structurally related to the Fujisawa compounds includes the basic phenylamidine moiety on one terminus and a central sulfonamide linkage.[11] Bradyzide, which evolved from high-throughput screening, has the prolylamide section of the Fournier compound and lipophilic benzodiazepine portion.[12]

However the structural features required for the bradykinin antagonist is not clear. From the bradykinin structure, it was generally accepted that the antagonist should have two basic groups separated by 10 Å, with a hydrophobic aromatic group;[5] even though the need for basic groups seems not to be absolute.[13]

In our search for the new bradykinin antagonists, we reported the bradykinin antagonistic effects of piperazine compounds derived from the anti-histamine drug, cetirizine.[14] Now we have designed and synthesized new compounds with piperazine, three amide bonds and a lipophilic ring system in each molecule. The solution-phase combinatorial synthesis route with iminodiacetic anhydride as template was employed for our discovery of nonpeptide bradykinin antagonists.[15,16]

1.1. Solution-phase combinatorial synthesis

To produce large numbers of diverse compounds rapidly and efficiently, a combinatorial synthesis using the iminodiacetic anhydride template was employed. The solution-phase, parallel synthesis of chemical libraries allowed the preparation of multi-milligram quantities of

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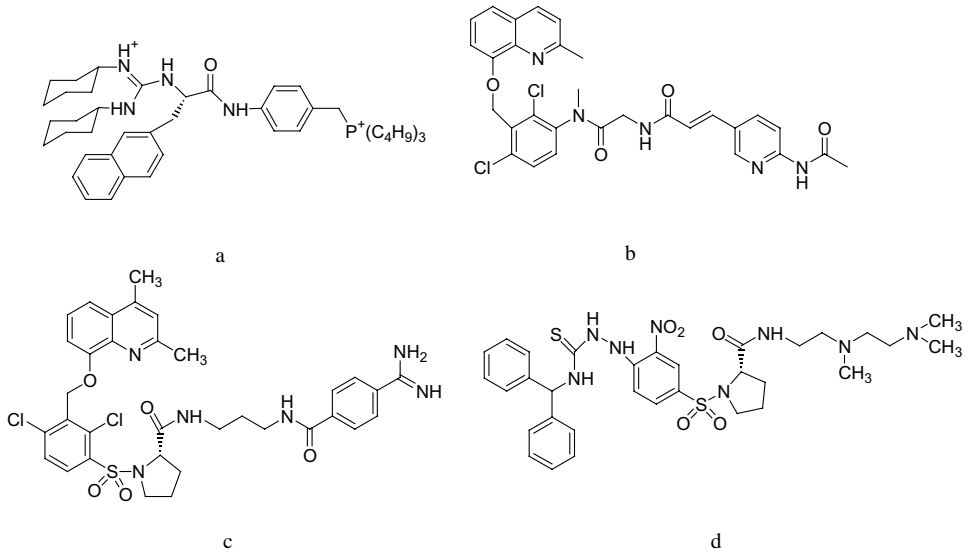
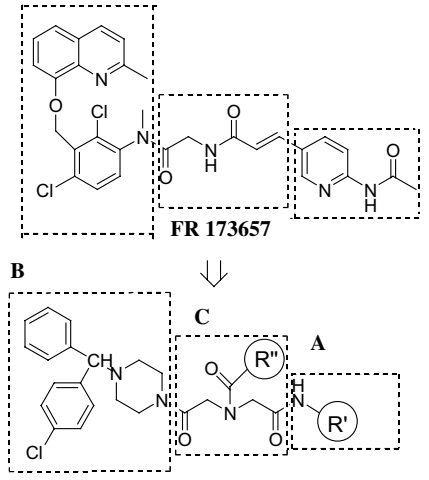


Figure 1. The structure of Win 64338 (a), FR 173657 (b), LF 16-0687 (c), and Bradyzide (d).

each individual member. The template contained three positions that could be sequentially functionalized enabling the synthesis of libraries with up to three variable units. Also in each step simple liquid/liquid extraction was used for both the isolation and purification of each intermediate and final product from the starting material, reactants, reagents, and their reaction by-products.

Each of the expected library members was obtained in a purified form in amounts ranging from 1 to 100 mg. In situ closure of *N*-Boc-iminodiacetic acid to the anhydride **1** followed by treatment with R^1NH_2 afforded the monoamides **AX**, which were purified by simple acid extraction to remove unreacted R^1NH_2, EDCI, and its reaction by-products. Each product was treated with 1-(4-chlorobenzhydryl)piperazine (**P**) and PyBOP to afford diamides **AXB1**, which were effectively purified by acid and base extractions to remove reaction by-products, the unreacted starting materials, and reagents. The Boc groups were removed using HCl in dioxane and the resulting secondary amines were coupled to carboxylic acids (R^3COOH) affording triamides **AXB1CX** using PyBrOP. The library consisted of individual compounds as a $10 \times 1 \times 5$ matrix affording 50 compounds. Each individual compound synthesized (intermediate and final product) was traditionally characterized by ^1H NMR and low-resolution FABMS (high-resolution FABMS for several compounds). Most of the final library products were suitable for direct use in screening efforts without further purification (Scheme 2).



Scheme 1. Design of the iminodiacetic acid triamide derivatives based on FR 173657.

2. Results and discussion

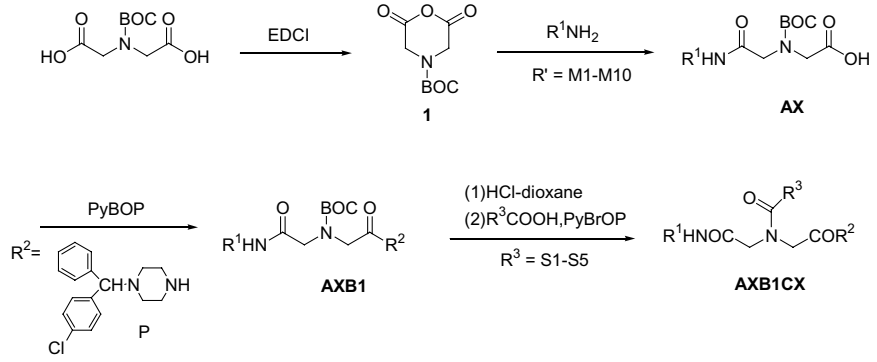
The structures of amines and acids used are illustrated in Table 1. The percent reaction yields are given in Table 2. The purity of selected final compounds was 80–90% in HPLC analysis using reported conditions.[16]

Each compound was tested for their bradykinin-induced contractilities on guinea-pig ileum smooth muscle in 0.1 μM concentration. As shown in Table 3, inhibitory activity of synthesized compounds to maximum contractility of bradykinin was varied from 0.4% to 46.3% ($n = 6$).

2.1. Antibradykinin effects

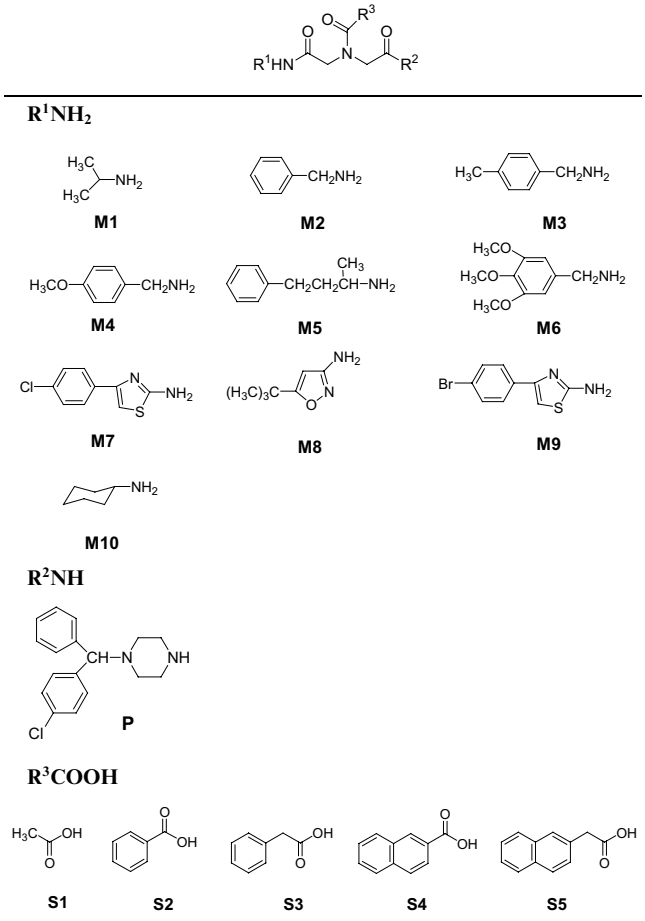
As shown in Scheme 1, FR 173657 and synthesized compounds can be considered as composed of three parts: terminal ring system (**A**), the aromatic rings (**B**), and amide bonds (**C**).

For the terminal (**A**) of the compounds, 10 amines (**M1**–**M10**) were employed. Because there was no structural feature for antibradykinin antagonist, some alkyl, or substituted aromatic amines were used. The compounds



Scheme 2. Solution-phase combinatorial synthesis route using the iminodiacetic anhydride template.

Table 1. The structures of amines and acids



with substituted benzyl groups (**A3**- and **A6**-series) showed good antibradykinin activities. But the compounds with simple alkyl groups, such as isopropyl (**A1**-series) and cyclohexyl (**A10**-series), exhibited poor activities. Compounds with heterocycles, such as isoxazole and thiazoles, showed poor activities, too.

To mimic the dichlorobenzene-linked terminal quinoline (**B**), 1-(4-chlorobenzhydryl)piperazine (**P**) was introduced in our synthesized compounds. In our previous

Table 2. The yield (%) of synthesized compounds

	C1	C2	C3	C4	C5
A1B1	NR[a]	40	64	45	26
A2B1	2	14	17	19	25
A3B1	64	66	74	85	82
A4B1	71	53	77	77	84
A5B1	76	77	34	97	89
A6B1	33	21	9	14	39
A7B1	13	53	99	61	47
A8B1	17	27	32	25	32
A9B1	20	21	23	34	22
A10B1	30	95	26	13	13

[a] No reaction.

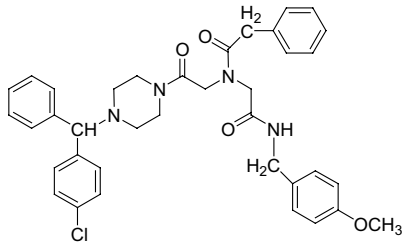
Table 3. Antibradykinin activity of synthesized compounds at 0.1 μM concentration in the guinea-pig ileum

Sample no	%Inhibition	Sample no	%Inhibition
A1B1C1	NR	**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
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
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
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
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
HOE 140	82.22 ± 2.31		

report[14] on the second-generation antihistamine agent cetirizine, the antibradykinin effects were observed from the compounds with 1-(4-chlorobenzhydryl)piperazine moiety. However the dual effect of antihistamine and antibradykinin could be expected.

For the substitution on the nitrogen of the middle amide bond (**C**) in our molecules, five acids (**S1**–**S5**) including naphthyl carboxylic acid adopted from structure of Win 64338 were employed. As the 2-naphthyl of Win 64338, this part was introduced for a distinct hydrophobic binding site in the B2 receptor since the aromatic residue in position 8 of bradykinin is known as an absolute requirement for high affinity binding. However when five different acids were introduced, little difference in antibradykinin activity was observed. Only the benzyl (**S3**) substitution showed slightly increased activity than the other compounds derived from different acids.

Among the 50 synthesized compounds, five compounds **A3B1C1**, **A3B1C3**, **A4B1C3**, **A5B1C5**, and **A6B1C3** showed high activity. The IC_{50} value of **A4B1C3** was ca. 10^{-7} M while that of the reference compound **HOE 140** was 10^{-8} M.[17] The potency of our compounds was less than the peptide antagonist **HOE 140** but the synthetic compounds has the merit of being nonpeptide. The potency (IC_{50}) of nonpeptide antagonists Win 64338 and FR 173657 has been reported as 3.9 μM and 0.56 nM, respectively, in a guinea-pig ileum binding affinity assay.[6] However the synthetic route for our compounds was very simple and conditions were very mild compared to Fujisawa compounds. The further optimization of these series of compounds by changing 1-(4-chlorobenzhydryl)piperazine (part B in Scheme 1) to various amines are under study.



A4B1C3

3. Conclusions

Fifty nonpeptide bradykinin B2 receptor antagonists were designed and synthesized by solution-phase combinatorial synthesis using the iminodiacetic anhydride template. The synthetic route for the preparation of the compounds was very simple and conditions were very mild. Most of the compounds synthesized showed inhibitory activity on bradykinin induced contraction at 0.1 μM concentration in the guinea-pig ileum. The potency was less than **HOE 140**, the peptide antagonist, but the synthetic compounds have the merit of being nonpeptide.

4. Experimental

4.1. General procedure for the first derivatization of *N*-Boc iminodiacetic acids

A mixture of *N*-((*tert*-butyloxy)carbonyl)iminodiacetic acid (2.13 mmol) and EDCI (2.13 mmol) in 6.4 mL of DMF was stirred for 1 h at room temperature (**A2**–**A10**) or at 50 °C (**A1**). Amine (**M1**–**M10**, 2.13 mmol) was added to the resulting solution via syringe while stirring (slightly exothermic). In reactions with aromatic amines (**M7**–**M9**) the catalyst DMAP (4.26 mmol, 2 equiv) were added to the resulting solution. The reaction mixture was stirred for 20 h before it was poured into a 250 mL separatory funnel. The product was diluted with EtOAc (60 mL), washed sequentially with 10% aqueous HCl (2×40 mL), and saturated aqueous NaCl (40 mL), dried over Na_2SO_4 and the solvent removed in vacuo to afford the products **A1**–**A10**.

4.1.1. *N*-((*tert*-Butyloxy)carbonyl)-*N*′-isopropyl iminodiacetic acid monoamide (A1). 0.31 g (53%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 3.9 (4H, m), 2.6 (1H, s), 1.3 (9H, s), 1.0 (6H, dd, $J = 6.4$ and 5.6 Hz); FABMS m/z 177.83 (100), 274.92 (34).

4.1.2. *N*-((*tert*-Butyloxy)carbonyl)-*N*′-benzyl iminodiacetic acid monoamide (A2). 0.52 g (75%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2 (5H, m), 4.4 (2H, m), 3.9–4.0 (4H, m), 1.3 and 1.4 (9H, two s); FABMS m/z 266.9 (100), 322.96 (62).

4.1.3. *N*-((*tert*-Butyloxy)carbonyl)-*N*′-(4-methylbenzyl)-iminodiacetic acid monoamide (A3). 0.49 g (68%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2 (4H, m), 4.4 (2H, m), 3.9–4.0 (4H, m), 2.3 (3H, s), 1.3 and 1.5 (9H, two s); FABMS m/z 57.5 (100), 336.95 (42).

4.1.4. *N*-((*tert*-Butyloxy)carbonyl)-*N*′-(4-methoxybenzyl)-iminodiacetic acid monoamide (A4). 0.43 g (55%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.1 (2H, m), 6.8 (2H, m), 4.3 (2H, d, $J = 5.6$ Hz), 3.9–4.0 (4H, m), 3.6 (3H, s), 1.4 (9H, two s); FABMS m/z 153.9 (100), 352.93 (24).

4.1.5. *N*-((*tert*-Butyloxy)carbonyl)-*N*′-(1-(methyl)-3-(phenyl)propyl)iminodiacetic acid monoamide (A5). 0.62 g (80%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.1–7.4 (5H, m), 4.1 (1H, m), 3.9–4.0 (4H, m), 2.6 (2H, m), 1.8 (2H, m), 1.4 (9H, two s), 1.2 (3H, d, $J = 7.2$ Hz); FABMS m/z 308.9 (100), 364.95 (58).

4.1.6. *N*-((*tert*-Butyloxy)carbonyl)-*N*′-(3,4,5-trimethoxybenzyl)iminodiacetic acid monoamide (A6). 0.23 g (26%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.5 (2H, m), 4.4 (2H, d, $J = 5.6$ Hz), 4.0 (4H, m), 3.8 (9H, s), 1.4 (9H, s); FABMS m/z 181.0 (100), 413.06 (22).

4.1.7. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(2-(4-(4-chlorophenyl)thiazole))iminodiacetic acid monoamide (A7). 0.29 g (32%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.9 (5H, m), 3.7–3.9 (4H, m), 1.2 and 1.3 (9H, two s); FABMS m/z 57.5 (100), 425.93 (26).

4.1.8. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(3-(5-*tert*-butylisoxazole))iminodiacetic acid monoamide (A8). 0.2 g (26%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.0(1H, s), 4.4(4H, s), 1.4(9H, s), 1.2 (9H, s); FABMS m/z 57.5 (100), 356.03 (18).

4.1.9. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(2-(4-(4-bromophenyl)thiazole))iminodiacetic acid monoamide (A9). 0.27 g (27%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.8 (5H, m), 3.7–3.9 (4H, s), 1.2 and 1.3 (9H, two s); FABMS m/z 73.3 (100), 469.84 (7).

4.1.10. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-cyclohexyliminodiacetic acid monoamide (A10). 0.3 g (12%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 4.0 (4H, m), 3.7 (1H, m), 1.6–1.9 (5H, m), 1.4 (9H, s), 1.2–1.4 (5H, m); FABMS m/z 153.9 (100), 315.02 (28).

4.2. General method for second diversification

The *N*-Boc-iminodiacetic monoamide (**A1**–**A10** each, 1.31 mmol, 1.0 equiv), 1-(4-chloro-benzhydryl)piperazine (**P**, 1.441 mmol, 1.1 equiv), and PyBOP (1.441 mmol, 1.1 equiv) were combined in a 30 mL vial. DMF (15 mL) was added followed by *i*-Pr_2NEt (2.62 mmol, 2.0 equiv). The reaction mixture was stirred 16 h at room temperature before it was poured into a 250 mL separatory funnel. The product was diluted with EtOAc (100 mL), washed sequentially with 10% aqueous HCl (3×50 mL), saturated aqueous $NaHCO_3$ (2×50 mL), and saturated aqueous NaCl (50 mL), dried (Na_2SO_4) and the solvent removed in vacuo to afford the products **A1B1**–**A10B1**.

4.2.1. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-isopropyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A1B1). 0.47 g (77%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.5 (9H, m), 4.3 (1H, s), 3.8–4.0 (4H, m), 3.6 (1H, s), 3.4–3.5 (4H, m), 2.2–2.3 (4H, m), 1.4 (9H, s), 1.3 (6H, dd, $J = 6.4$ and 13.2 Hz); FABMS m/z 200.9 (100), 543.02 (18).

4.2.2. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-benzyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A2B1). 0.61 g (73%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.5 (14H, m), 4.4 (3H, m), 4.2 (2H, d, $J = 5.6$ Hz), 3.8 (2H, d, $J = 18$ Hz), 3.5–3.6 (4H, m), 2.3–2.5 (4H, m), 1.4 (9H, two s); FABMS m/z 201.0 (100), 591.3 (12.5).

4.2.3. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(4-methylbenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A3B1). 0.73 g (86%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–7.6 (13H, m), 4.4 (1H, s), 4.3 (2H, d, $J = 6$ Hz), 4.2 (2H, d, $J = 4.8$ Hz), 3.8 (2H, d, $J = 18.4$ Hz), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m), 2.2 (3H, s), 1.3 (9H, two s); FABMS m/z 176.0 (100), 605.20 (15).

4.2.4. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(4-methoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A4B1). 0.67 g (89%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.6 (11H, m), 6.8 (2H, m), 4.4 (1H, s), 4.3 (2H, d, $J = 5.6$ Hz), 4.2 (2H, d, $J = 3.2$ Hz), 3.8 (2H, d, $J = 20.4$ Hz), 3.7 (3H, s), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m), 1.4 (9H, two s); FABMS m/z 201.0 (100), 621.34 (14).

4.2.5. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(1-(methyl)-3-(phenyl)propyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A5B1). 0.78 g (94%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.1–7.5 (14H, m), 4.4 (1H, s), 4.2–4.3 (2H, m), 3.9 (1H, m), 3.7–3.8 (2H, m), 3.5–3.7 (4H, m), 2.5–2.7 (2H, m), 2.3–2.5 (4H, m), 1.7 (2H, m), 1.4 (9H, two s), 1.0–1.2 (3H, dd, $J = 6.8$ and 16.4 Hz); FABMS m/z 201.0 (100), 633.24 (32).

4.2.6. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(3,4,5-trimethoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A6B1). 0.31 g (81%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.5 (9H, m), 6.6 (2H, s), 4.4 (1H, s), 4.3 (2H, d, $J = 6.0$ Hz), 4.2 (2H, d, $J = 11.6$ Hz), 4.0 (2H, d, $J = 7.2$ Hz), 3.8 (9H, s), 3.5–3.6 (4H, m), 2.3–2.5 (4H, m), 1.3 (9H, two s); FABMS (Na) m/z 132.9 (100), 703.28 (15).

4.2.7. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(2-(4-(4-chlorophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A7B1). 0.34 g (69%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–8.0 (14H, m), 4.45 (1H, s), 4.4 (2H, m), 4.1–4.15 (2H, m), 3.6–3.8 (4H, m), 2.4–2.5 (4H, m), 1.3 and 1.4 (9H, two s); FABMS m/z 132.9 (100), 694.11 (10.5).

4.2.8. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(3-(5-*tert*-butylisoxazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A8B1). 0.29 g (83%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.6 (9H, m), 6.6 (1H, s), 4.4 (1H, s), 4.3 (2H, d, $J = 5.2$ Hz), 4.0 (2H, d, $J = 14$ Hz), 3.5–3.7 (4H, m), 2.4–2.5 (4H, m), 1.4 (9H, two s), 1.3 (9H, two s); FABMS m/z 200.9 (100), 624.18 (46).

4.2.9. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-(2-(4-(4-bromophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A9B1). 0.23 g (92%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.9 (14H, m), 4.45 (1H, s), 4.4 (2H, d, $J = 4.0$ Hz), 4.1 (2H, d, $J = 20$ Hz), 3.6–3.8 (4H, m), 2.4–2.5 (4H, m), 1.3 and 1.4 (9H, two s); FABMS m/z 73.4 (100), 738.14 (3.3).

4.2.10. *N*-((*tert*-Butyloxy)carbonyl)-*N′*-cyclohexyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid diamide (A10B1). 0.47 g (65%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.9 (9H, m), 4.4 (1H, s), 4.2 (2H, d, $J = 2.8$ Hz), 3.7 (2H, d, $J = 15.2$ Hz), 3.65 (1H, m), 3.5–3.6 (4H, m), 2.3–2.5 (4H, m), 1.5–1.8 (5H, m), 1.4 (9H, two s), 1.2–1.3 (5H, m); FABMS m/z 200.9 (100), 583.16 (60).

4.3. General procedure for the third diversification

$CHCl_3$ (1 mL) and 4 M HCl–dioxane (1 mL) were added to **A1B1**–**A10B1** (0.017 mmol) in a 4 mL vial and this mixture was allowed to stand for 3 h. The solvent and excess acid were removed under stream of N_2. The resulting residue was dissolved in 1 mL of DMF and added *i*-Pr_2NEt (0.055 mmol). After dissolution carboxylic acid (**S1**–**S5**, 0.019 mmol, 1.1 equiv) was added, followed by PyBrOP (0.017 mmol, 1 equiv). After stirring for 16 h, the reaction mixture was diluted with EtOAc (40 mL) and washed with 10% aqueous HCl (3×30 mL), saturated aqueous $NaHCO_3$ (2×30 mL), and saturated aqueous NaCl (30 mL), dried ($NaSO_4$), and concentrated to afford the products **A2B1C1**–**A10B1C5**.

4.3.1. *N*-Acetyl-*N′*-benzyl-*N″*-1-(4-chlorobenzhydryl)-piperazine iminodiacetic acid triamide (A2B1C1). 1 mg (2%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.5 (14H, m), 4.4 (3H, m), 4.2 (2H, d, $J = 5.6$ Hz), 3.8 (2H, d, $J = 18$ Hz), 3.5 (4H, m), 2.4–2.5 (4H, m), 2.0 (3H, s); FABMS m/z 148.9 (100), 533.02 (3.8).

4.3.2. *N*-Acetyl-*N′*-(4-methylbenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A3B1C1). 58 mg (64%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–7.5 (13H, m), 4.6 (1H, s), 4.2–4.4 (4H, m), 3.9–4.0 (2H, m), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m), 2.3 (3H, s), 2.0 (3H, s); FABHRMS (NBA) m/z 547.2475 (M^+H, $C_{31}H_{36}O_3N_4Cl$ requires 547.2476).

4.3.3. *N*-Acetyl-*N′*-(4-methoxybenzyl)-*N″*-1-(4-chlorobenhydryl)piperazine iminodiacetic acid triamide (A4B1C1). 11.9 mg (71%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.8–7.5 (13H, m), 4.4 (1H, s), 4.1–4.3 (4H, m), 3.8 (2H, s), 3.7 (3H, s), 3.4–3.5 (4H, m), 2.2–2.3 (4H, m), 1.9 (3H, s); FABMS m/z 154.0 (100), 563.05 (3.4).

4.3.4. *N*-Acetyl-*N′*-(1-(methyl)-3-(phenyl)propyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A5B1C1). 23 mg (76%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.1–7.5 (14H, m), 4.5 (1H, s), 4.3–4.4 (4H, m), 4.2 (1H, s), 3.5–3.7 (4H, m), 2.5–2.7 (2H, m), 2.3–2.5 (4H, m), 2.0 (3H, s) 1.7–1.8 (2H, m), 1.0–1.2 (3H, dd, $J = 6.4$ and 25.2 Hz); FABMS m/z 200.9 (100), 575.05 (30).

4.3.5. *N*-Acetyl-*N′*-(3,4,5-trimethoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A6B1C1). 7.3 mg (33%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.5 (9H, m), 6.6 (2H, m), 4.4 (1H, s), 4.2–4.4 (4H, m), 3.9–4.1 (2H, m), 3.8 (9H, s), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m), 2.0 (3H, s); FABMS m/z 73.3 (100), 623.14 (1.0).

4.3.6. *N*-Acetyl-*N′*-(2-(4-(4-chlorophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A7B1C1). 10.0 mg (13%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.8 (14H, m), 4.3 (1H, s), 4.1–4.2 (4H, m), 3.4–3.5 (4H, m), 2.4 (4H, m), 2.0 (3H, s); FABMS m/z 148.9 (100), 635.96 (12).

4.3.7. *N*-Acetyl-*N′*-(3-(5-*tert*-butylisoxazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A8B1C1). 20.0 mg (17%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.6 (9H, m), 6.6 (1H, s), 4.6 (1H, s), 4.0–4.4 (4H, m), 3.6–3.7 (4H, m), 2.4–2.5 (4H, m), 2.0 (3H, s), 1.3–1.4 (9H, s); FABMS m/z 55.5 (100), 566.02 (15).

4.3.8. *N*-Acetyl-*N′*-(2-(4-(4-bromophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A9B1C1). 40.0 mg (20%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–7.7 (14H, m), 4.2 (1H, s), 4.0–4.2 (4H, m), 3.4–3.6 (4H, m), 2.4 (4H, m), 2.0 (3H, s); FABMS m/z 55.5 (100), 679.92 (2.5).

4.3.9. *N*-Acetyl-*N′*-cyclohexyl-*N″*-1-(4-chlorobenzhydryl)-piperazine iminodiacetic acid triamide (A10B1C1). 10.0 mg (30%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.9 (9H, m), 4.4 (1H, s), 4.2–4.4 (4H, m), 3.8 (1H, s), 3.5–3.6 (4H, m), 2.5 (4H, m), 2.0 (3H, s), 1.8 (5H, m), 1.2 (5H, m); FABMS (Na) m/z 441.2 (100), 547.08 (5.4).

4.3.10. *N*-Benzoyl-*N′*-isopropyl-*N″*-1-(4-chlorobenzhydryl)-piperazine iminodiacetic acid triamide (A1B1C2). 11 mg (40%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.5 (14H, m), 4.4 (1H, s), 4.2–4.4 (4H, m), 4.1 (1H, s), 3.5–3.6 (4H, m), 2.3–2.5 (4H, m), 0.9–1.1 (6H, m); FABMS m/z 148.9 (100), 547.05 (1.9).

4.3.11. *N*-Benzoyl-*N′*-benzyl-*N″*-1-(4-chlorobenzhydryl)-piperazine iminodiacetic acid triamide (A2B1C2). 7 mg (14%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.8 (19H, m), 4.4 (3H, m), 4.0–4.3 (4H, m), 3.5–3.7 (4H, m), 2.3–2.5 (4H, m); FABMS m/z 201.0 (100), 595.20 (15).

4.3.12. *N*-Benzoyl-*N′*-(4-methylbenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A3B1C2). 67 mg (66%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–7.5 (18H, m), 4.4 (1H, s), 4.3–4.4 (4H, m), 4.0–4.2 (2H, m), 3.5–3.6 (4H, m), 2.4–2.5 (4H, m), 2.2 (3H, s); FABHRMS (NBA) m/z 609.2630 (M^+H, $C_{36}H_{38}O_3N_4Cl$ requires 609.2632).

4.3.13. *N*-Benzoyl-*N′*-(4-methoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A4B1C2). 9 mg (53%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.8–7.5 (18H, m), 4.4 (1H, s), 4.1–4.4 (4H, m), 3.8–4.0 (2H, m), 3.7 (3H, s), 3.4–3.5 (4H, m), 2.2–2.4 (4H, m); FABHRMS (NBA) m/z 647.2401 (M^+Na, $C_{36}H_{37}O_4N_4ClNa$ requires 647.2401).

4.3.14. *N*-Benzoyl-*N′*-(1-(methyl)-3-(phenyl)propyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A5B1C2). 37 mg (77%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.6 (19H, m), 4.5 (1H, s), 4.3–4.4 (2H, m), 4.0 (1H, s), 3.9–4.0 (2H, m), 3.5–3.7 (4H, m), 2.5–2.7 (2H, m), 2.2–2.5 (4H, m), 1.7–1.8 (2H, m), 1.0–1.2 (3H, dd, $J = 6.4$ and 16.4 Hz); FABMS m/z 200.9 (100), 637.03 (30).

4.3.15. *N*-Benzoyl-*N′*-(3,4,5-trimethoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A6B1C2). 5 mg (21%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.6 (14H, m), 6.6 (2H, s), 4.4 (1H, s), 4.2–4.4 (4H, m), 3.9–4.1 (2H, m), 3.8 (9H, s), 3.5–3.6 (4H, m), 2.2–2.4 (4H, m); FABMS (Na) m/z 132.8 (100), 707.06 (0.55).

4.3.16. *N*-Benzoyl-*N′*-(2-(4-(4-chlorophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A7B1C2). 35.0 mg (53%)^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–8.0 (19H, m), 4.4 (1H, s), 4.2–4.3 (4H, m), 3.5–3.7 (4H, m), 2.3–2.4 (4H, m); FABMS m/z 105.1 (100), 698.03 (24.2).

4.3.17. *N*-Benzoyl-*N′*-(3-(5-*tert*-butylisoxazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A8B1C2). 40.0 mg (27%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–8.0 (14H, m), 6.6 (1H, s), 4.5 (1H, s), 4.2–4.4 (4H, m), 3.4–3.6 (4H, m), 2.4–2.5 (4H, m), 1.4 (9H, s); FABMS m/z 200.9 (100), 628.16 (48).

4.3.18. *N*-Benzoyl-*N′*-(2-(4-(4-bromophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A9B1C2). 40.0 mg (21%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.9 (19H, m), 4.3 (1H, s), 4.1–4.3 (4H, m), 3.5–3.7 (4H, m), 2.3–2.5 (4H, m); FABMS m/z 154.0 (100), 741.95 (4).

4.3.19. *N*-Benzoyl-*N′*-cyclohexyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A10B1C2). 35 mg (95%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.9 (14H, m), 4.4 (1H, s), 4.0–4.4 (4H, m), 3.8 (1H, s), 3.5–3.6 (4H, m), 2.4–2.6 (4H, m), 1.8–1.9 (2H, s), 1.6–1.8 (5H, m), 1.5 (5H, m); FABMS m/z 132.8 (100), 587.22 (15).

4.3.20. *N*-Phenylacetyl-*N′*-isopropyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A1B1C3). 6 mg (64%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.5 (14H, m), 4.4 (1H, s), 4.2–4.4 (4H, m), 3.65 (1H, m), 3.6 (2H, s), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m), 0.9–1.1 (6H, m); FABMS m/z 149.0 (100), 561.14 (2.3).

4.3.21. *N*-Phenylacetyl-*N′*-benzyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A2B1C3). 6 mg (17%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.6 (19H, m), 4.6 (1H, s), 4.0–4.4 (6H, m), 3.6 (2H, m), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m); FABMS m/z 201.0 (100), 609.21 (18).

4.3.22. *N*-Phenylacetyl-*N′*-(4-methylbenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A3B1C3). 77 mg (74%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–7.5 (18H, m), 4.6 (1H, s), 4.3–4.4 (4H, m), 4.0–4.1 (2H, m), 3.6 (2H, s), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m), 2.3 (3H, s); FABHRMS (NBA) m/z 623.2786 (M^+H, $C_{37}H_{40}O_3N_4Cl$ requires 623.2789).

4.3.23. *N*-Phenylacetyl-*N′*-(4-methoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A4B1C3). 25 mg (77%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.8–7.5 (18H, m), 4.4 (1H, s), 4.2 (4H, m), 3.8–4.0 (2H, m), 3.7 (3H, s), 3.6 (2H, s), 3.4–3.5 (4H, m), 2.2–2.3 (4H, m); FABHRMS (NBA) m/z 661.2560 (M^+Na, $C_{37}H_{39}O_4N_4ClNa$ requires 661.2558).

4.3.24. *N*-Phenylacetyl-*N′*-(1-(methyl)-3-(phenyl)propyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A5B1C3). 14 mg (34%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–7.4 (19H, m), 4.5 (1H, s), 4.2–4.3 (2H, m), 3.9 (1H, m), 3.7–3.8 (2H, m), 3.4–3.5 (4H, m), 3.6 (2H, s), 2.4–2.6 (2H, m), 2.1–2.3 (4H, m), 1.5–1.7 (2H, m), 0.9–1.0 (3H, dd, $J = 6.8$ and 22.4 Hz); FABMS m/z 200.9 (100), 651.15 (20).

4.3.25. *N*-Phenylacetyl-*N′*-(3,4,5-trimethoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A6B1C3). 2 mg (9%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.5 (14H, m), 6.6 (2H, s), 4.6 (1H, s), 4.2–4.4 (4H, m), 3.9–4.1 (2H, m), 3.8 (9H, s), 3.7 (2H, s), 3.5–3.6 (4H, m), 2.3–2.5 (4H, m); FABMS m/z 148.9 (100), 699.1 (10).

4.3.26. *N*-Phenylacetyl-*N′*-(2-(4-(4-chlorophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A7B1C3). 80 mg (99%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.8 (14H, m), 7.0–7.2 (5H, m), 4.2 (1H, s), 4.0–4.2 (4H, m), 3.6 (2H, s), 3.4–3.5 (4H, m), 2.3–2.4 (4H, m); FABMS m/z 419.1 (100), 711.95 (16.2).

4.3.27. *N*-Phenylacetyl-*N′*-(3-(5-*tert*-butylisoxazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A8B1C3). 44 mg (32%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.6 (14H, m), 6.6 (1H, s), 4.6 (1H, s), 4.0–4.4 (4H, m), 3.8 (2H, s), 3.5–3.7 (4H, m), 2.4–2.5 (4H, m), 1.4 (9H, s); FABMS m/z 73.3 (100), 642.14 (14).

4.3.28. *N*-Phenylacetyl-*N′*-(2-(4-(4-bromophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A9B1C3). 50.0 mg (23%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.1–7.7 (19H, m), 4.2 (1H, s), 4.0–4.2 (4H, m), 3.6 (2H, s), 3.4–3.6 (4H, m), 2.3–2.4 (4H, m); FABMS m/z 200.9 (100), 755.98 (11).

4.3.29. *N*-Phenylacetyl-*N′*-cyclohexyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A10B1C3). 10 mg (26%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.8 (14H, m), 4.3 (1H, s), 4.0–4.2 (4H, m), 3.9 (1H, s), 3.7 (2H, s), 3.6 (4H, m), 2.4–2.5 (4H, m), 1.6–1.8 (5H, m), 1.2 (5H, m); FABMS m/z 148.9 (100), 601.00 (3.2).

4.3.30. *N*-(2-Naphthoyl)-*N′*-isopropyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A1B1C4). 6 mg (45%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–8.0 (16H, m), 4.4 (1H, s), 4.2–4.3 (4H, m), 4.0 (1H, s), 3.5–3.7 (4H, m), 2.2–2.5 (4H, m), 0.9–1.1 (6H, m); FABMS m/z 149.0 (100), 597.15 (2.0).

4.3.31. *N*-(2-Naphthoyl)-*N′*-benzyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A2B1C4). 8 mg (19%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.3–8.0 (21H, m), 4.4 (3H, m), 3.8–4.2 (4H, m), 3.4–3.6 (4H, m), 2.4–2.5 (4H, m); FABMS m/z 201.0 (100), 645.2 (15).

4.3.32. *N*-(2-Naphthoyl)-*N′*-(4-methylbenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A3B1C4). 93 mg (85%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–8.0 (20H, m), 4.5 (1H, s), 4.3–4.4 (4H, m), 4.0 (2H, s), 3.5–3.7 (4H, m), 2.3–2.5 (4H, m), 1.8 (3H, s); FABHRMS (NBA) m/z 659.2787 (M^+H, $C_{40}H_{40}O_3N_4Cl$ requires 659.2789).

4.3.33. *N*-(2-Naphthoyl)-*N′*-(4-methoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A4B1C4). 29 mg (77%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.8–8.0 (20H, m), 4.4 (1H, s), 4.2–4.3 (4H, m), 3.9 (2H, s), 3.7 (3H, s), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m); FABMS m/z 57.5 (100), 675.24 (23).

4.3.34. *N*-(2-Naphthoyl)-*N′*-(1-(methyl)-3-(phenyl)propyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A5B1C4). 43 mg (97%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–8.0 (21H, m), 4.4 (1H, s), 4.2–4.4 (2H, m), 4.0 (1H, s), 3.8–3.9 (2H, m), 3.3–3.6 (4H, m), 2.5–2.6 (2H, m), 2.2–2.4 (4H, m), 1.6–1.7 (2H, m), 1.0–1.2 (3H, dd, $J = 6.8$ and 23.6 Hz); FABMS m/z 200.9 (100), 687.15 (18.2).

4.3.35. *N*-(2-Naphthoyl)-*N′*-(3,4,5-trimethoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A6B1C4). 4 mg (14%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–8.0 (16H, m), 6.6 (2H, s), 4.5 (1H, s), 4.2–4.4 (4H, m), 4.0–4.2 (2H, m), 3.8 (9H, s), 3.5–3.6 (4H, m), 2.4–2.5 (4H, m); FABMS m/z 132.8 (100), 735.22 (2.0).

4.3.36. *N*-(2-Naphthoyl)-*N′*-(2-(4-(4-chlorophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A7B1C4). 60 mg (61%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–8.0 (21H, m), 4.4 (1H, s), 4.2–4.3 (4H, m), 3.6–3.8 (4H, m), 2.4–2.5 (4H, m); FABMS m/z 419.2 (100), 748.03 (11.5).

4.3.37. *N*-(2-Naphthoyl)-*N′*-(3-(5-*tert*-butylisoxazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A8B1C4). 40.0 mg (25%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.3–8.2 (16H, m), 6.6 (1H, s), 4.5 (1H, s), 3.9–4.3 (4H, m), 3.5–3.7 (4H, m), 2.4–2.5 (4H, m), 1.3 (9H, s); FABMS m/z 154.9 (100), 678.00 (11.2).

4.3.38. *N*-(2-Naphthoyl)-*N′*-(2-(4-(4-bromophenyl)thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A9B1C4). 40.0 mg (21%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–8.0 (21H, m), 4.4 (1H, s), 4.1–4.3 (4H, m), 3.4–3.6 (4H, m), 2.4 (4H, m); FABMS m/z 155.0 (100), 791.92 (3.18).

4.3.39. *N*-(2-Naphthoyl)-*N′*-cyclohexyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A10B1C4). 80 mg (34%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.3–8.2 (16H, m), 4.4 (1H, s), 4.2–4.3 (4H, m), 3.9 (1H, s), 3.4–3.6 (4H, m), 2.4–2.5 (4H, m), 1.8–1.9 (5H, m), 1.6 (5H, m); FABMS m/z 149.0 (100), 637.28 (6.8).

4.3.40. *N*-(2-Naphthylacetyl)-*N′*-isopropyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A1B1C5). 7 mg (26%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.8 (16H, m), 4.4 (1H, s), 4.2–4.3 (4H, m), 3.8 (1H, m), 3.55 (2H, s), 3.4–3.5 (4H, m), 2.2–2.4 (4H, m), 0.9–1.1 (6H, m); FABMS m/z 154.0 (100), 611.18 (1.35).

4.3.41. *N*-(2-Naphthylacetyl)-*N′*-benzyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A2B1C5). 8 mg (25%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.5 (21H, m), 4.6 (1H, s), 4.4 (4H, m), 4.0–4.2 (2H, m), 3.8 (2H, s), 3.5–3.6 (4H, m), 2.3–2.5 (4H, m); FABMS m/z 201.0 (100), 659.2 (10.5).

4.3.42. *N*-(2-Naphthylacetyl)-*N′*-(4-methylbenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A3B1C5). 91 mg (82%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–7.9 (20H, m), 4.6 (1H, s), 4.3–4.4 (4H, m), 4.0–4.2 (2H, m), 3.8 (2H, s), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m), 2.3 (3H, s); FABHRMS (NBA) m/z 673.2943 (M^+H, $C_{41}H_{42}O_3N_4Cl$ requires 673.2945).

4.3.43. *N*-(2-Naphthylacetyl)-*N′*-(4-methoxybenzyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A4B1C5). 34 mg (84%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 6.8–7.9 (20H, m), 4.4 (1H, s), 4.2–4.3 (4H, m), 3.9–4.1 (2H, m), 3.8 (2H, s), 3.7 (3H, s), 3.5 (2H, s), 3.4–3.5 (4H, m), 2.1–2.3 (4H, m); FABMS m/z 200.9 (100), 689.01 (19.2).

4.3.44. *N*-(2-Naphthylacetyl)-*N′*-(1-(methyl)-3-(phenyl)-propyl)-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A5B1C5). 40 mg (90%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.0–7.8 (21H, m), 4.4 (1H, s), 4.1–4.3 (2H, m), 4.0 (1H, m), 3.7–3.8 (2H, m), 3.4–3.6 (4H, m), 2.7 (2H, s), 2.4–2.6 (2H, m), 2.1–2.3 (4H, m), 1.5–1.7 (2H, m), 1.0–1.2 (3H, t, $J = 6.4$ Hz); FABMS m/z 200.9 (100), 701.13 (17).

4.3.45. *N*-(2-Naphthylacetyl)-*N′*-(3,4,5-trimethoxybenzyl)-*N″*-1-(4-chloro-benzhydryl)piperazine iminodiacetic acid triamide (A6B1C5). 10 mg (39%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.9 (16H, m), 6.6 (2H, s), 4.6 (1H, s), 4.3–4.4 (4H, m), 4.0–4.2 (2H, m), 3.8 (9H, s), 3.7 (2H, s), 3.5–3.6 (4H, m), 2.3–2.4 (4H, m); FABMS (Na) m/z 132.8 (100), 771.18 (24.2).

4.3.46. *N*-(2-Naphthylacetyl)-*N′*-(2-(4-(4-chlorophenyl)-thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A7B1C5) . 60 mg (47%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.8 (21H, m), 4.3 (1H, s), 4.1–4.2 (4H, m), 3.8 (2H, s), 3.4–3.5 (4H, m), 2.3–2.4 (4H, m); FABMS m/z 201.0 (100), 762.11 (18.2).

4.3.47. *N*-(2-Naphthylacetyl)-*N′*-(3-(5-*tert*-butylisoxazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A8B1C5). 44 mg (32%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.9 (16H, m), 6.6 (1H, s), 4.4 (1H, s), 3.9–4.1 (4H, m), 3.8 (2H, s), 3.5–3.7 (4H, m), 2.4–2.5 (4H, m), 1.3 (9H, s); FABMS m/z 57.5 (100), 692.10 (36).

4.3.48. *N*-(2-Naphthylacetyl)-*N′*-(2-(4-(4-bromophenyl)-thiazole))-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A9B1C5). 60 mg (22%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 7.2–7.9 (21H, m), 4.7 (1H, s), 4.0–4.5 (4H, m), 3.8 (2H, s), 3.5–3.6 (4H, m), 2.4–2.5 (4H, m); FABMS m/z 73.3 (100), 805.82 (2.05).

4.3.49. *N*-(2-Naphthylacetyl)-*N′*-cyclohexyl-*N″*-1-(4-chlorobenzhydryl)piperazine iminodiacetic acid triamide (A10B1C5). 6 mg (13%); ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.0 (16H, m), 4.4 (1H, s), 4.0–4.3 (4H, m), 3.9 (1H, s), 3.8 (2H, s), 3.5–3.6 (4H, m), 2.4 (4H, m), 1.8–1.9 (5H, m), 1.6 (5H, m); FABMS m/z 73.3 (100), 651.02 (3.8).

4.4. Bradykinin-induced contractions of guinea-pig ileum

Bradykinin, captopril, indomethacin, dithiothreitol, atropine, and **HOE 140** were obtained from Sigma (St. Louis, MO, USA). All peptides were dissolved and diluted in physiological saline (0.9%). Captopril, dithiothreitol and atropine were dissolved in DMSO and diluted in physiological saline. Indomethacin and compounds **A2B1C1**–**A10B1C5** were dissolved and diluted in DMSO. The composition of the Tyrode solution was 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 bath was less than 0.1% and had no effect on the tissue's responsiveness to bradykinin.

Male Hartley guinea-pigs weighing 250–600 g (Jaeil, Korea) were fasted overnight and decapitated. At a level 2 cm above the ileocecal junction, a section of ileum approximately 40 cm in length was removed and placed in warm (37 °C) Tyrode solution. Strips of muscle, 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 BK (1 μM), every 20-min interval, to assay sensibility and reproducibility of the contractile response.[18] Only segments producing reproducible responses were used. The last control response was taken as 100% and subsequent results obtained with bradykinin antagonists expressed as a percentage of this. The segments were incubated with the bradykinin antagonists (0.1 μM) for 5 min before BK was added.

To minimize degradation of bradykinin and to prevent responses due to neuronal activation or prostaglandin production, Tyrode solutions contained 1 μM each of captopril, atropine, dithiothreitol, and indomethacin. In control tissues a corresponding volume of physiological saline was applied in place of the antagonist.

4.5. Statistical analysis

Pair-wise comparisons were done by the one-tailed Student's t test. Probability values $p < 0.05$ were considered to be statistically significant. All results are expressed as the mean ± SEM ($n = 6$), 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.

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