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Antihistamine Activities of Iminodiacetamide Derivatives

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Abstract – A series of *N,N'*-substituted iminodiacetamide derivatives was synthesized and evaluated their inhibitory effects on the histamine-induced smooth muscle contraction in guinea-pig ileum and on the histamine release from IgE-sensitized RBL-2H3 cells (rat basophilic leukemia cell line). Compounds **A3**, **A4** and **A5** which have 1-(4-chlorobenzhydryl) piperazine moiety, showed both moderate antihistamine activity and histamine release inhibitory activity.

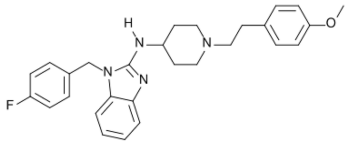
Keywords: Iminodiacetamide, Allergy, Antihistamine, Guinea-pig ileum, Histamine release, RBL-2H3

INTRODUCTION

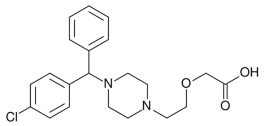
Histamine has an important role in inflammation, gastric acid secretion and as a neurotransmitter. During inflammation, histamine is released from mast cells and basophils. Among the four different histamine receptors, H_1, H_2, H_3 and H_4, most pathophysiological effects of histamine during allergic disease and immediate hypersensitivity response are derived from H_1 receptor stimulation (Thurmond *et al*., 2008; Parsons and Ganellin, 2006).

H_1 receptor antagonists are widely used in the treatment of allergic disorders. In addition to their histamine H_1 receptor blocking capabilities, some histamine H_1 receptor antagonists that influence allergic/inflammatory responses by affecting the mediator release from mast cells are known (Rimmer and Church, 1990; Church, 2001; Gelfand *et al*., 2004, Marshall, 2000, Passalacqua *et al*., 2002, Baroody and Naclerio, 2000). These include astemizole, loratadine and azelastine (Berthon *et al*., 1994; Nabe *et al*., 1989; Lau and Pearce, 1990; Chand *et al*., 1985). Many of these drugs have amphiphilic properties, and their effects on membrane structures are responsible for the inhibition of the mediator release (Fischer *et al*., 1995).

A series of compounds with two amide bonds and various lipophilic ring systems, *N,N'*-substituted iminodiacetamide derivatives was synthesized and their antihistamine activities were evaluated. Most of the synthesized compounds have the modified structure from classical H_1 receptor antagonists such as certirizine and astemizole, which include 1-(4-chlorobenzhydryl) piperazine moiety or 4-methoxybenzyl moiety and modified –C-C-N linkage with aromatic ring structures on either side of the two acid amide chains.



Astemizole



Cetirizine

The compounds were tested for their inhibitory effects on the histamine-induced smooth muscle contraction in guinea-pig ileum at 0.1 μM and on the histamine release from IgE-sensitized RBL-2H3 cells at 10 μM concentration.

MATERIALS AND METHODS

Benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonum-hexafluorophosphate (PyBOP) and 4-dimethylaminopyridine (DMAP) were purchased from Novabiochem (La Jolla, CA, USA) and the other reagents were obtained from Aldrich Chemical Company (Milwaukee, WI, USA).

The ^{1}H NMR spectra were recorded on a Bruker Avance 400MHz Varian FT-NMR spectrometer, and trimethylsilane (TMS) was used as an internal standard. Mass spectra were obtained on a Tandem Mass Spectrometer JMS-HX110/110A (Jeol, Japan). High and low resolution mass spectra were performed using the FAB technique. HPLC analysis was performed on a HPLC Agilent 1100

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series (Hewlett Packard, USA). DMF and CH_3OH were HPLC grade.

Synthesis by the use of iminodiacetic anhydride template

To produce a large number of diverse compounds rapidly and efficiently, the iminodiacetic anhydride template was used as reported in our previous study (Kam *et al*., 2004). The simple liquid/liquid extraction method for both isolation and purification of each intermediate and final product by Boger (Cheng *et al*., 1996) was adopted (Figure 1). Structures of synthesized compounds are presented in Table 1.

Synthesis of *N*-[(*tert*-butyloxy)carbonyl]iminodiacetic acid (2)

A 1000 mL flask was charged with iminodiacetic acid (**1**, 13.3 g, 100 mmol), dioxane (200 mL) and NaOH (8 g, 200 mmol) dissolved in 200 mL of water. When a homogeneous solution was formed, di-*tert*-butyldicarbonate (25 mL, 110 mmol) was added in portions. After stirring at 25°C for 72 h, the reaction mixture was washed with diethylether (2 × 100 mL) and the aqueous layer was acidified with the 10% aqueous HCl (100 mL). This was extracted with ethyl acetate (3 × 150 mL), and the combined organic layers were washed with saturated NaCl solution (2 × 150 mL), and dried over Na_2SO_4.

Synthesis of *N*-substituted iminodiacetic acid monoamides ($5_{A\text{-}C}$)

A mixture of *N*-[(tert-butyloxy)carbonyl]iminodiacetic acid (**2**, 2.13 mmol, 1 equiv.) and EDCI (2.13 mmol, 1 equiv.) in 6.4 mL of DMF was stirred for 1 h at room temperature. Amine (**A-C** each, 2.13 mmol, 1 equiv.) was added, and the mixture was stirred for 20 h. The reaction mixture was poured into a 250 mL separatory funnel, diluted with ethyl acetate (60 mL), washed sequentially with 10% aqueous HCl (2 × 40 mL) and saturated aqueous NaCl (40 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo* to afford the products, *N*-BOC-iminodiacetic monoamides ($\mathbf{4_{A\text{-}C}}$).

In a 4 ml vial 4 M HCl in dioxane (2.5 mL) was added to $\mathbf{4_{A\text{-}C}}$ (each, 0.017 mmol), and the reaction mixture was stirred for 3 h. The removal of solvent and excess acid gave the final products, *N*-substituted iminodiacetic acid monoamides ($\mathbf{5_{A\text{-}C}}$).

Synthesis of *N, N'*-substituted iminodiacetic acid diamides (A1-C24)

The derivatives $\mathbf{4_{A\text{-}C}}$ (each, 1.31 mmol, 1.0 equiv.), amine (**1-24** each, 1.441 mmol, 1.1 equiv.) and PyBOP (1.441 mmol, 1.1 equiv.) were combined in a 30 ml vial, and DMF (15 ml) was added followed by *i*-Pr_2NEt (2.62 mmol, 2.0 equiv.). The reaction mixture was stirred for 16 h at room temperature before it was poured into a 250 mL separatory funnel. The product was diluted with ethyl acetate (100 mL), washed sequentially with 10% aqueous HCl (3 × 50 mL), saturated aqueous $NaHCO_3$ (2 × 50 mL), and saturated aqueous NaCl (50 mL), and dried over Na_2SO_4. The solvent was removed *in vacuo* to afford the products, *N*-BOC-*N',N''*-substituted iminodiacetic acid diamides ($\mathbf{6_{A1\text{-}C24}}$).

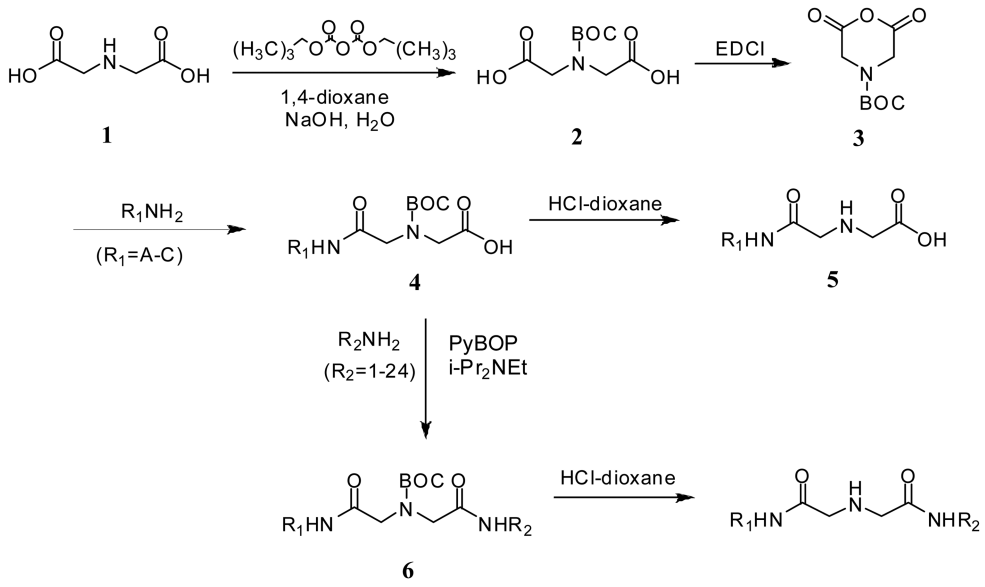
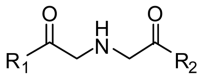


Fig. 1. Synthesis of iminodiacetic acid diamides using iminodiacetic anhydride template

In a 4 ml vial 4 M HCl in dioxane (2.5 mL) was added to $\mathbf{6}_{\mathbf{A1\text{-}C24}}$ (each, 0.017 mmol), and this mixture was allowed to stirred for 3 h. The removal of solvent and excess acid gave the final products, *N,N'*-substituted iminodiacetic acid diamides (**A1-C24)**.

Table I. Structures of synthesized compounds



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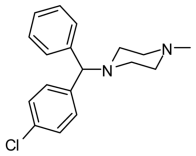
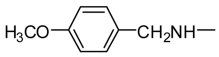
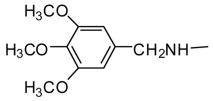
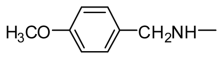
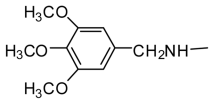
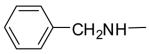
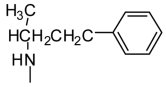
		

R2:			
			
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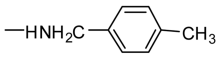	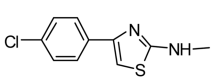	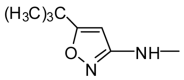	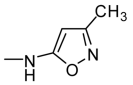
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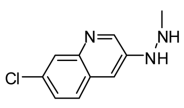	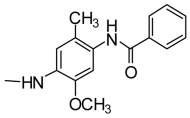	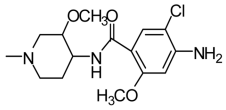	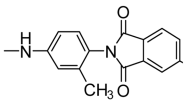
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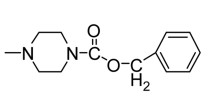	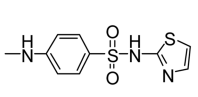	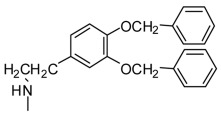	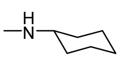
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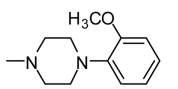	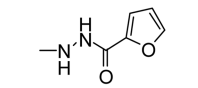	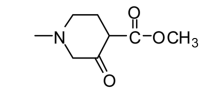	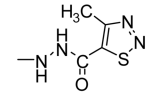
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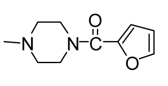	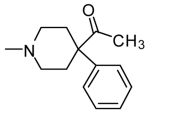	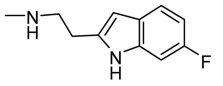	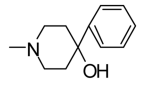
21	**22**	**23**	**24**

Table II. Yield, Formulation and High and Low Resolution Mass Spectral Data for Synthesized Compounds

Compound	Yield (%)	Formulation	Mass(calcd)	Mass (observed)	Elemental composition(M^++H)
5A	78	yellow oil	402.1584	402.1582	$C_{21}H_{25}ClN_3O_3$
5B	55	pale brown solid	253.1188	253.1192	$C_{12}H_{17}N_2O_4$
5C	100	yellow solid	313.1400	313.1400	$C_{14}H_{21}N_2O_6$
A1	67	yellow solid	521.2319	521.2316	$C_{29}H_{34}ClN_4O_3$
A3	99	yellow solid	491.2214	491.2214	$C_{28}H_{32}ClN_4O_2$
A4	100	yellow solid	533.2683	533.2685	$C_{31}H_{38}ClN_4O_2$
A5	96	yellow solid	505.2370	505.2372	$C_{29}H_{34}ClN_4O_2$
A7	98	yellow solid	524.0543	524.06(50%)[a]	$C_{28}H_{35}ClN_5O_3$
A8	99	brown solid	481.9745	481.95(40%)[a]	$C_{25}H_{29}ClN_5O_3$
A15	99	yellow solid	717.2948	717.00(30%)[a]	$C_{43}H_{46}ClN_4O_4$
A16	66	yellow solid	483.2527	483.2528	$C_{27}H_{36}ClN_4O_2$
B1	25	white solid	372.1923	372.1924	$C_{20}H_{26}N_3O_4$
B2	62	white solid	432.2135	432.2129	$C_{22}H_{30}N_3O_6$
B6	57	pale brown solid	445.1101	445.1097	$C_{21}H_{22}N_4O_3ClS$
B7	90	yellow oil	375.2032	375.2029	$C_{19}H_{27}N_4O_4$
B9	92	yellow solid	428.1489	428.1489	$C_{21}H_{23}N_5O_3Cl$
B11	100	yellow solid	548.2276	548.2278	$C_{26}H_{35}N_5O_6Cl$
B12	99	yellow solid	521.1592	521.1592	$C_{27}H_{26}N_4O_5Cl$
B17	93	pink solid	427.2345	427.2348	$C_{23}H_{31}N_4O_4$
B18	100	yellow solid	361.1512	361.1512	$C_{17}H_{21}N_4O_5$
B20	100	yellow solid	393.1345	393.1344	$C_{16}H_{21}N_6O_4S$
B21	100	yellow oil	415.1981	415.1981	$C_{21}H_{27}N_4O_5$
B22	100	brown crystalline	438.2393	438.2395	$C_{25}H_{32}N_3O_4$
B24	100	yellow brown solid	412.2236	412.2238	$C_{23}H_{30}N_3O_4$
C2	99	white solid	492.2346	492.2343	$C_{24}H_{34}N_3O_8$
C6	100	yellow oil	505.1312	505.1312	$C_{23}H_{26}N_4O_5ClS$
C10	90	white solid	551.2506	551.2502	$C_{29}H_{35}N_4O_7$
C11	100	yellow solid	608.2487	608.2487	$C_{28}H_{39}N_5O_8Cl$
C12	100	yellow solid	581.1803	581.1802	$C_{29}H_{30}N_4O_7Cl$
C13	97	yellow crystalline	516.2506	515.2502	$C_{26}H_{35}N_4O_7$
C14	100	yellow oil	550.1430	550.1432	$C_{23}H_{28}N_5O_7S_2$
C18	100	yellow solid	443.1543	443.1541	$C_{19}H_{24}N_4O_7Na$
C19	100	yellow crystalline	451.4703	452.00(62%) [a]	$C_{21}H_{30}N_3O_8$
C20	96	yellow solid	453.1556	453.1555	$C_{18}H_{25}N_6O_6S$
C21	95	yellow solid	475.2193	475.2191	$C_{23}H_{31}N_4O_7$
C22	84	brown crystalline	498.2604	498.2605	$C_{27}H_{36}N_3O_6$
C23	78	yellow solid	473.2200	473.2195	$C_{24}H_{30}N_4O_5F$
C24	83	yellow solid	472.2448	472.2452	$C_{25}H_{34}N_3O_6$

a. Low resolution mass spectral data with corresponding percentage

Measurement of histamine-induced smooth muscle contraction in guinea-pig ileum

Guinea-pig ileum contraction by histamine was measured by the previously reported methods (Akah *et al*., 1997; Merlos *et al*., 1997, Kam *et al*., 2005). The composition of the Tyrode solution was NaCl 136.9 mM, KCl 2.7 mM, $CaCl_2$ 1.8 mM, $MgCl_2$ 1.15 mM, NaH_2PO_4 0.4 mM, $NaHCO_3$ 11.9 mM, and glucose 5.6 mM. Male Hartley guinea-pigs weighing 290~400 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 and aerated with 95% O_2 / 5% CO_2. Tissue contractions were recorded isometrically on a Grass model 76E polygraph. After 60 minutes of equilibration period, a stable baseline tone was reached, and two or three contractions were obtained in response to histamine (1 μM), every 20 minutes, to assay the sensibility and reproducibility of the contractile response. The last control response was taken as 100%, and subsequent contractions obtained with the test compounds were expressed as a percentage of this response. The segments were incubated with the test compounds (0.1 μM) for 15 min before histamine was added. To minimize degradation of histamine and prevent responses due to neuronal activation or prostaglandin production, Tyrode solution contained 1 μM each of captopril, atropine, dithiothreitol, and indomethacin (Sigma Chemical Co., St. Louis, MO, USA).

Measurement of histamine-release from IgE-sensitized RBL-2H3 cells

Preparation of the RBL-2H3 cells

RBL-2H3 cells were obtained from the American Type Culture Collection. RBL-2H3 cells were cultured in Minimum Essential Medium (MEM) with Earle's salts and L-glutamine (Invitrogen Co., NY, USA) that was supplemented with 15% fetal bovine serum (FBS, Invitrogen Co., NY, USA) and 1% penicillin/streptomycin (PS, Invitrogen Co., NY, USA) in a 100 cm^2 dish in a humidified atmosphere of 5% CO_2 at 37°C. When the RBL-2H3 cells were 80-90% confluent, the cells were washed twice with 5 mL of phosphate buffer solution (PBS , NaCl 4 g, KCl 0.1 g, KH_2PO_4 0.1 g, Na_2HPO_4 0.575 g in 500 ml) and detached with trypsin (0.05%) - EDTA (0.02%) solution for 5 minutes at 37°C for serial passage. For the histamine release assay, RBL-2H3 cells were seeded into 24-well culture plates (6.25×10^4 cells/ml) in 0.5 mL medium for each well. Cells were incubated overnight at 37°C and sensitized with 0.2 μg/ml of rat IgE kappa myeloma (IgE, Serotec Ltd., Oxford, UK).

In vitro histamine release assay

The effects of compounds on the histamine release from RBL-2H3 cells were measured by the previously reported methods (Nakatani *et al*., 2002; Ikawati *et al*.,

Table III. ^{1}H NMR peak assignments for synthesized compounds measured in DMSO-d_6 at 400MHz

Compound	^{1}H NMR assignments @400MHz (δ ppm)
5A	δ 7.63-7.64 (4H,m), 7.3-7.4 (5H,m), 4.01 (2H,s), 3.76 (2H,s), 3.51 (8H,s)
5B	δ 7.15 (2H,d,*J*=8.8 Hz), 6.84 (2H,d,*J*=8.8 Hz), 4.22 (2H,d,*J*=6.0 Hz), 3.83 (2H,s), 3.72 (2H,s), 3.68 (3H,s)
5C	δ 6.60 (2H,s), 4.28 (2H,d,*J*=5.6 Hz), 3.86 (2H,s), 3.78 (2H,s), 3.77 (6H, s), 3.63 (3H,s)
A1	δ 7.2-7.6(11H,m),6.7-6.8 (2H,m),4.4 (1H,s),4.3 (2H,d,*J*=5.6Hz), 4.2 (2H,d, *J*=3.2Hz), 3.8 (2H,d,*J*=20.4Hz), 3.7 (3H,s), 3.5-3.6 (4H,m), 2.3-2.4(4H,m)
A3	δ 7.2-7.5 (14H,m), 4.4 (1H,s), 4.38 (2H,d,*J*=6.0 Hz), 4.2 (2H,d,*J*=6.0 Hz), 3.8 (2H,d,*J*=18.0 Hz), 3.5-3.6 (4H,m), 2.3-2.5 (4H,m)
A4	δ 7.1-7.5 (14H,m), 4.4 (1H,s), 4.2-4.3 (2H,m), 3.85-3.95 (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), 1.6-1.7 (2H,m), 1.1 (1H,t,*J*=7.0 Hz), 1.1 (1H,d,*J*=6.8 Hz), 1.1 (1H,d,*J*=6.8 Hz)
A5	δ 7.0-7.6 (13H,m), 4.4 (1H,s), 4.3 (2H,d,*J*=6.0 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)
A7	δ 7.2-7.6 (9H,m), 6.6 (1H,s), 4.4 (1H,s), 4.3 (2H,d,*J*=5.2 Hz), 3.9-4.0 (2H,d, *J*=14.4 Hz), 3.5-3.7 (4H,m), 2.4 (4H,m), 1.25 (9H,two s)
A8	δ 7.2-7.6 (9H,m), 6.2 (1H,d,*J*=3.2Hz), 4.5 (4H,s), 4.4 (2H,d,J=9.2 Hz), 4.0 (2H, d,*J*=10.0 Hz), 3.6-3.8 (4H,m), 2.4-2.5 (4H,m), 2.2 (3H,d,*J*=5.2 Hz)
A15	δ 6.6-7.4 (22H,m), 5.0-5.2 (4H,m), 4.2 (1H,s), 3.9-4.0 (2H,m), 3.6-6.8 (2H,m), 3.4-3.5 (4H,m), 3.2-3.3 (4H,m), 2.2-2.3 (4H,m)
A16	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), 3.6-3.65 (1H,m), 3.5-3.6 (4H,m), 2.3-2.5 (4H,m), 1.5-1.8 (5H,m), 1-1.3 (5H,m)

Table III. ^{1}H NMR peak assignments for synthesized compounds measured in DMSO-d_6 at 400MHz

B1	δ7.2(4H,d,*J*=8.8Hz),6.9(4H,d,*J*=8.8Hz),4.26(4H,d,*J*=5.6Hz),3.8(4H,s),3.7(6H,s)
B2	δ 7.15 (2H,d,*J*=8.8 Hz), 6.83 (2H,d,*J*=8.8 Hz), 6.55 (2H,s), 4.21 (4H,d,*J*= 5.6 Hz), 3.75 (2H,s), 3.72 (2H,s), 3.70 (6H,s), 3.67 (3H,s), 3.56 (3H,s)
B6	δ 7.91 (2H,d,*J*=8.8 Hz), 7.78 (1H,s), 7.50 (2H,d,*J*=8.8 Hz), 7.22 (2H,d,*J*=8.8 Hz),6.89(2H,d,*J*=8.8Hz),4.29(2H,d,*J*=5.6Hz),4.13(2H,s),3.9(2H,s),3.72(3H,s)
B7	δ 7.2 (2H,d,*J*=8.4 Hz), 6.7 (2H,d,*J*=8.8 Hz), 6.5 (1H,s), 4.5 (2H,s), 4.3 (4H, s), 4.2(2H,m), 3.6 (3H,s), 1.2 (9H,s)
B9	δ 8.72 (1H,d,*J*=5.2 Hz), 8.62 (1H,d,*J*=9.2 Hz), 8.13 (1H,s), 7.87 (1H,d,*J*= 9.2), 7.23 (2H,d,*J*=8.8 Hz), 7.04 (1H,d,*J*=6.8 Hz), 6.90 (2H,d,*J*=8.8 Hz), 4.30 (2H,d,*J*=5.6 Hz), 4.12 (2H,s), 3.88 (2H,s), 3.73 (3H,s)
B11	δ 7.73 (1H,s), 7.22 (2H,d,*J*=8.8 Hz), 6.90 (2H,d,*J*=8.8 Hz), 6.50 (1H,s), 4.28 (2H,d,*J*=5.6 Hz), 4.15 (2H,s), 3.85 (3H,s), 3.73 (3H,s), 3.56 (2H,s), 3.36 (2H, s), 2.49-2.51 (4H,m), 2.08 (2H,s)
B12	δ 8.0 (1H,m), 7.9 (2H,m), 7.6 (1H,m), 7.5 (1H,m), 7.3 (1H,d,*J*=8.4 Hz), 7.2 (2H,d,*J*=8.8 Hz), 6.9 (2H,d,*J*=8.8 Hz), 4.3 (2H,d,*J*=5.6 Hz), 4.0 (2H,s), 3.8 (2H,s), 3.7 (3H,s), 2.1 (3H,s)
B17	δ 7.2-7.23 (2H,m), 6.96-7 (2H,m), 6.9-6.92 (2H,m), 6.88-6.89 (2H,m), 4.28 (2H,d,*J*=5.6Hz), 4.1-4.1(2H,m), 3.8 (3H,s), 3.75-3.77 (2H,m), 3.7(3H,s), 3.63-3.67 (4H,m), 2.9-3 (4H,m)
B18	δ 7.92 (1H,s), 7.27 (1H,s), 7.22 (2H,d,*J*=8.8 Hz), 6.90 (2H,d,*J*=8.8 Hz), 6.67-6.68 (1H,m), 4.28 (2H,d,*J*=6 Hz), 3.93 (2H,s), 3.8 (2H,s), 3.73 (3H,s)
B20	δ 7.20-7.23 (2H,m), 6.87-6.91 (2H,m), 4.27-4.28 (2H,m), 3.97 (2H,s), 3.84 (2H, s), 3.73 (3H,s), 3.56 (3H,s)
B21	δ 7.77-7.78 (1H,m), 7.31 (2H,d,*J*=8.8 Hz), 7.03-7.04 (1H,m), 6.89 (2H,d,*J*=8.8 Hz), 6.60-6.62 (1H,m), 4.34 (2H,s), 4.26 (2H,s), 3.95 (2H,s), 3.84-3.85 (2H,m), 3.79-3.80 (2H,m),3.77 (3H,s), 3.68-3.7 (2H,m), 3.6-3.62 (2H,m)
B22	δ 7.39-7.43 (2H,m), 7.33-7.36 (2H,m), 7.29-7.31 (1H,m), 7.21 (2H,d,*J*=8.8 Hz), 6.89 (2H,d, *J*=8.8 Hz), 4.27 (2H,d, *J*=5.6 Hz), 4.07 (2H,s), 3.83-3.86 (2H,m), 3.73 (3H,s), 3.51(1H,s), 3.14-3.26(2H,m), 2.36-2.39(2H,m), 1.9-2.0(2H,m), 1.92 (3H,s)
B24	δ 7.61 (1H,d,*J*=8.0 Hz), 7.34-7.47 (3H,m), 7.29 (1H,d,*J*=7.6 Hz), 7.20-7.23 (2H, m), 6.88-6.91 (2H, m), 4.34-4.39 (1H, m), 4.26-4.29 (2H, m), 4.08-4.17 (3H, m), 3.74-3.76 (2H, m), 3.73 (3H, s), 3.56 (6H, s)
C2	δ 6.60 (4H,s), 4.27 (4H,d,*J*=5.6 Hz), 3.83 (4H,s), 3.75 (12H,s), 3.62 (6H,s)
C6	δ 7.9 (2H,d,*J*=8.8Hz), 7.6(1H,s),7.4(2H,d,*J*=8.8Hz),6.7(1H,s),6.68(1H,s),4.75 (1H,s),4.4(1H,s),4.3(1H,s),4.2(1H,s),4.1(1H,s),3.8(6H,d,*J*=4.8Hz),3.7 (3H,s)
C10	δ 7.98 (2H,d,*J*=7.2 Hz), 7.76 (1H,s), 7.57-7.60 (1H,m), 7.51-7.55 (2H,m), 7.1 (1H,s), 6.6(2H,s), 4.30 (2H,d,*J*=5.6 Hz), 4.07 (2H,s), 3.89 (2H,s), 3.80 (3H, s), 3.77 (6H,s), 3.63 (3H,s), 2.16 (3H,s)
C11	δ7.73(1H,s),6.6(2H,s),6.5(1H,s),4.29(2H,d,*J*=5.6Hz),4.13(2H,s),3.8(3H,s),3.77 (6H,s),3.6(3H,s), 3.56(2H,s),3.41(2H,s),3.36(2H,s),2.49-2.50(4H,m),2.1(2H,s)
C12	δ 7.9 (3H,m), 7.8(1H,m), 7.7 (1H,m), 7.2 (1H,dd,*J*=8.4 Hz,3.2 Hz), 6.7(2H,d, *J*=8.4 Hz), 4.6 (2H,s),4.4(2H,s), 4.4(2H,s), 3.75(6H,s), 3.6 (3H,s), 2.1 (3H, s)
C13	δ 7.33-7.42(5H,m), 6.7 (2H,s), 5.1 (2H,s), 4.35 (2H,s),4.2 (2H,s), 3.96 (2H,s), 3.8(6H,s),3.7(3H,s), 3.6(2H,s), 3.51-3.58(4H,m), 3.49-3.5(2H, m),2.5(2H, m)
C14	δ 7.81-7.86 (4H,m), 7.19-7.20 (1H,d,*J*=4.8Hz), 6.78-6.79 (1H,d,*J*=4.8 Hz), 6.68 (1H,s), 6.64 (1H,s), 4.35 (2H,d,*J*=4.8 Hz), 4.16-4.17 (2H,m), 3.99 (1H, s), 3.94 (1H,s), 3.81 (6H,s), 3.80 (3H,s)
C18	δ 7.92 (1H,s), 7.27 (1H,s), 6.68 (1H,s), 6.61 (2H,s), 4.28-4.29 (2H,d,*J*=5.6 Hz), 3.95 (2H,s), 3.86 (2H,s), 3.76 (6H,s), 3.62 (3H,s)
C19	δ 6.62 (2H,s), 4.28 (2H,d,*J*=5.6 Hz), 4.18 (1H,s), 4.14 (1H,s), 4.00 (1H,s), 3.8 (1H,s),3.77(6H,s),3.76(3H,s),3.66-3.7(2H,m),3.6(3H,s),3.56(9H,s),3.5(1H,m)
C20	δ 6.61 (2H,s), 4.29 (2H,d,*J*=5.6 Hz), 3.98 (2H,s), 3.88 (2H,s), 3.76 (6H,s), 3.75 (3H,s), 3.63 (3H,s)
C21	δ 7.80 (1H,s), 7.04 (1H,s), 6.73 (2H,s), 6.62 (1H,s), 4.36 (2H,s), 4.26 (2H,s), 3.95 (2H,s), 3.83 (6H,s), 3.68 (3H,s), 3.59 (8H,s)
C22	δ 7.38-7.45 (4H,m), 7.31-7.34 (1H,m), 6.71 (2H,s), 4.34 (2H,s), 4.17-4.18 (2H,m), 3.96-3.98 (2H,m), 3.91 (2H,s), 3.82 (6H,s), 3.68 (3H,s), 3.54 (1H,s), 3.34-3.36 (2H,m), 3.22-3.24 (2H,m), 2.43-2.46 (2H,m), 1.95 (3H,s)
C23	δ 7.50-7.53 (1H,m), 7.18 (1H,s), 7.10-7.13 (1H,m), 6.82-6.87 (1H,m), 6.62 (2H,s), 4.28 (2H,d,J=5.6 Hz), 3.83 (2H,s), 3.77 (6H,s), 3.74 (1H,s), 3.63 (3H,s), 2.82-2.86 (2H,m)
C24	δ 8.0 (1H,d,J=7.6 Hz), 7.8-7.86 (3H,m), 7.8 (1H,d,J=7.6 Hz), 7.17 (2H,s), 6.54 (1H,s), 4.76-4.80 (2H,m), 4.2 (6H,m), 4.1 (3H,m), 4.03-4.04 (8H,m), 3.65-3.67 (1H,m), 2.90-2.94 (1H,m), 2.72-2.78 (1H,m), 2.61-2.66 (1H,m)

2001; Miyamoto *et al*., 1995). After sensitizing the cells with IgE, the medium was removed, and the cells were washed twice with 0.5 mL of MEM and preincubated in the absence or presence of the compounds at 10 μM concentration at 37°C for 15 min. For comparison, cetirizine (10 μM) was used as a standard for inhibitory effect of antihistamine drug. RBL-2H3 cells were stimulated with 12.5 μg/mL of Mouse anti-rat-IgE heavy chain (anti-IgE, Serotec Ltd., Oxford, UK) as antigen for 30 min, and histamine released into the medium was measured by automated fluorometric procedure as described by Siraganian (Siraganian, 1975; 1974). For the *in vitro* quantitative determination of histamine, the Astoria™ analyzer, series 300 system, was used.

The histamine release from RBL-2H3 cells was expressed as percentage of the stimulated histamine release without drug after subtracting the spontaneous release. The spontaneous histamine release is the histamine release in the absence of anti-IgE. The calculation is as follows:

$$\text{Histamine Release(\%)} = \frac{\text{Histamine relesae with drug-Spontaneous release}}{\text{Histamine release without drug-Spontaneous relesae}} \times 100$$

RESULTS AND DISCUSSIONS

Table IV. Inhibition of the histamine-induced smooth muscle contraction in guinea-pig ileum at 0.1 μM concentration and effect on the histamine release from RBL-2H3 cells at 10 μM concentration

Compound	Contraction inhibition (%)[a]	Histamine release (%)[b]	Log P[c]
5A	13.9±5.4	80.7±8.9	2.12
5B	16.1±6.1	79.9±6.4	-0.27
5C	17.4±6.8	86.0±18	-0.52
A1	31.1±5.0	76.2±16	3.31
A3	26.4±5.8	55.1±12	3.44
A4	30.5±7.2	66.3±23	4.45
A5	20.7±5.4	61.2±24	3.93
A7	18.3±4.9	76.0±27	4.02
A8	9.28±2.1	109±3.9	2.43
A15	17.9±3.6	88.3±34	6.93
A16	26.7±5.3	122±34	3.25
B1	8.98±2.3	93.7±11	0.92
B2	9.80±1.2	106±21	0.67
B6	16.2±1.8	113±26	3.36
B7	16.3±2.8	53.6±2.0	1.63
B9	11.9±1.9	117±4.8	1.14
B11	7.81±0.9	112±9.4	-0.22
B12	14.2±2.5	103±23	2.64
B17	7.68±1.0	78.0±26	1.26
B18	11.3±1.2	116±1.6	-0.94
B20	11.3±0.7	51.3±32	NC[d]
B21	12.0±3.0	73.7±16	-0.91
B22	13.3±3.0	75.9±20	1.19
B24	10.4±2.7	94.3±11	0.6
C2	8.36±1.4	98.3±2.8	0.42
C6	14.4±1.8	50.1±11	3.10
C10	12.7±1.9	103±4.6	1.89
C11	11.2±1.8	67.6±16	-0.47
C12	10.5±1.6	109±21	2.39
C13	14.4±1.7	89.3±6.0	0.64
C14	13.3±1.0	71.9±21	0.85
C18	11.7±2.2	112±3.9	-1.19
C19	17.0±2.0	112±0.7	-1.54
C20	9.99±1.2	91.1±11	NC[d]
C21	11.2±2.6	78.3±19	-1.16
C22	13.6±2.3	61.9±53	0.94
C23	11.5±1.8	82.2±6.0	0.66
C24	13.1±3.9	75.4±6.0	0.34
Cetirizine	65.7±1.5	85.8±10	3.11

a. The results are expressed as the mean ± S.E.M. of at least four determinations.
b. The results are expressed as the mean ± S.E.M. of at least two experiments performed in triplicate (n≥6).
c. Log P values were calculated using CS ChemDraw Ultra software (version 5.0 (1989), Cambridge, USA).
d.NC : Not calculated.

Synthesis

Thirty eight compounds of *N,N'*-substituted iminodiacetamide compounds with diverse functional moieties were synthesized (Table 1). All the final products were confirmed by ^{1}H NMR and high and low resolution FABMS (Tables 2 and 3). Without optimization, most of the final products were obtained in 11-154 mg quantities (25-100% yields, Table 2) and used as samples for screening efforts without further purification. The purity of the final products was 80-95% in HPLC analysis based on the reported conditions (Boger *et al*., 1999).

Inhibition of histamine-induced smooth muscle contraction in guinea-pig ileum

The synthesized compounds were tested for their inhibitory effects on the histamine-induced smooth muscle contraction in guinea-pig ileum at 0.1 μM concentration. As shown in Table 4, the inhibitory activities of 0.1

μM synthesized compounds to maximum contractility of 1 μM histamine varied from 8 to 31% (n=4~9).

All three *N*-substituted iminodiacetic acid monoamides (**5_{A-C}**) showed similar antihistamine activities to each other. *N*,*N'*-substituted iminodiacetic acid diamides (**A1-C24**) showed differences in their antihistamine activities depending on the two functional moieties. Compounds **A1** and **A4**, which have 1-(4-chlorobenzhydryl) piperazine moiety (**A**) in one side chain, and 4-methoxybenzyl (**1**) and 4-methybenzyl (**4**) groups on the other side chain, showed more than 30% inhibition of the smooth muscle contraction in the guinea-pig ileum. In addition, compounds **A3** and **A16** with benzyl and cyclohexyl groups showed the moderate inhibition of histamine-induced smooth muscle contraction. On the contrary, when 4-methoxybenzyl (**B**) and/or 3,4,5-trimethoxybenzyl (**C**) moieties were incorporated at both side chains, the percentage of inhibition was less than 10%. When other functional groups such as piperidine benzamide (**11**) or thiadiazole carbohydrazide (**20**) were introduced in **B** and **C** series, the inhibition was also reduced. Thus, 1-(4-chlorobenzhydryl) piperazine group was suggested to be more important for the antihistamine activity than other functional groups.

Effects on the histamine release from IgE-sensitized RBL-2H3 cells

The synthesized compounds were screened for their effects on the histamine release from IgE-sensitized RBL-2H3 cells at 10 μM concentration. Histamine release from IgE-sensitized RBL-2H3 cells was induced by anti-IgE as antigen stimulation. The percent of the histamine release from RBL-2H3 cells affected by the synthesized compounds is shown in Table 4, which ranged between 50%~122% of the histamine release.

The compounds showed differences in their effects on the histamine release from the IgE-sensitized RBL-2H3 cells depending on the two functional groups. Compounds **A3**, **A4,** and **A5**, which showed moderate inhibition in smooth muscle contraction assay, also exhibited the significant inhibition of the histamine release, in addition to compounds with *tert*-butylisoxazole (**B7**), thiadiazole carbohydrazide (**B20**), and chlorophenyl thiazole (**C6**) groups.

For several anti-allergic drugs, it has been suggested that their effect on mast cells is mediated by the interaction with cell membrane (Fischer *et al*., 1995). As lipophilicity is an important property for the affinity and interaction with cell membrane, we investigated the relationship between partition coefficients and effects on the histamine release. The estimation of logarithm of partition coefficient, log P, was calculated with CS ChemDraw Ultra software (version 5.0 (1989), Cambridge, USA) and is also presented in Table 4. For compounds containing 1-(4-chlorobenzhydryl) piperazine moiety (**A**), favorable log P values for inhibiting exocytosis were 3-5. The high value of log P suggested that the compounds were located in lipophilic cell compartments like membranes and hydrophobic cores of proteins (Fischer *et al*., 1995). However, for compounds containing 4-methoxybenzyl moiety (**B**) and/or 3,4,5-trimethoxybenzyl moiety (**C**), favorable log P values for the inhibitory effects were 1-3, and high intracellular concentrations were related to their activities (Dearden, 1990). Because the synthesized compounds have diverse functional groups which can affect the partition coefficient, they can also act via different mechanisms and this can affect several membrane related processes.

In conclusion, compounds **A1-5** showed moderate antihistamine activities in guinea-pig smooth muscle contraction as well as inhibition of histamine release. These compounds have 1-(4-chlorobenzhydryl) piperazine moiety and give structural information to be active for the treatment of allergies and inflammatory diseases.

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