A New Class of Nonpeptide Bradykinin B_2 Receptor Ligand, Incorporating a 4-Aminoquinoline Framework. Identification of a Key Pharmacophore To Determine Species Difference and Agonist/Antagonist Profile

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Introduction of various aliphatic amino groups at the 4-position of the quinoline moiety of our nonpeptide bradykinin (BK) B_2 receptor antagonists afforded highly potent ligands for human B_2 receptor with various affinities for guinea pig B_2 receptor, indicating remarkable species difference. A representative 4-dimethyamino derivative **40a** exhibited subnanomolar and nanomolar binding affinities for human and guinea pig B_2 receptors, respectively, and significantly inhibited BK-induced bronchoconstriction in guinea pigs at 10 μg/kg by intravenous administration. Further chemical modification led us to discover unique partial agonists for the human B_2 receptor that increase inositol phosphates (IPs) production by themselves in Chinese hamster ovary (CHO) cells expressing the cloned human B_2 receptor. Although their potency and efficacy were much lower than those of BK, we identified them as screening leads for nonpeptide B_2 agonists. In these studies it was revealed the 4-substituent of the quinoline moiety is the key pharmacophore to determine species difference and agonist/antagonist profiles.

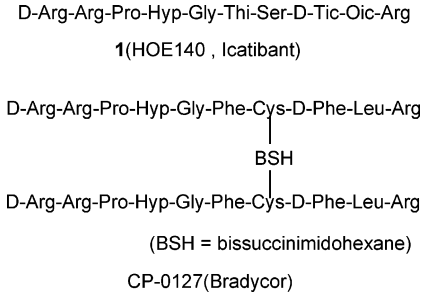
Introduction

Human kinins consist of two endogenous peptides, bradykinin (BK; Arg[1]-Pro[2]-Pro[3]-Gly[4]-Phe[5]-Ser[6]-Pro[7]-Phe[8]-Arg[9]) and kallidin (KD; [Lys[0]]BK; Lys[1]-Arg[2]-Pro[3]-Pro[4]-Gly[5]-Phe[6]-Ser[7]-Pro[8]-Phe[9]-Arg[10]). There are at least two subtypes of specific cell surface receptors, designated as B_1 and B_2, both of which have been identified by molecular cloning and pharmacological means.[1−3] Kinins are highly potent agonists of the B_2 receptor which is expressed constitutively in many tissues and is thought to mediate most of the biological actions of BK.[1,3] The B_2 receptor is coupled with G-protein which stimulates phosphatidylinositol (PI) hydrolysis.

BK exhibits highly potent and diverse proinflammatory activities and is believed to play important roles in a variety of inflammatory diseases.[4] Recently, it was also suggested that BK may be involved in the pathology of small cell lung cancer (SCLC),[5,6] bacterial and viral infections,[7,8] and Alzheimer's disease.[9] Therefore, the development of specific BK antagonists has been of great importance for investigating the pathophysiological roles of BK and for developing a novel class of therapeutic drugs.

Since 1985 a number of peptide B_2 antagonists have been synthesized,[10−14] including the clinically evaluated second generation antagonists, **1** (HOE140; icatibant; [D-Arg[0],Hyp[3],Thi,[5]D-Tic[7],Oic[8]]BK) and CP0127 (bradycor) (Chart 1). On the other hand, in 1993 WIN64338 was disclosed as the first nonpeptide B_2 antagonist.[15] However, it was reported to be not so selective[16] and to be practically inactive against B_2 receptors in isolated human umbilical vein.[17] In 1996 we presented **3a** (FR 173657) as the first potent, selective, and orally active nonpeptide B_2 receptor antagonist.[18] Since then, we have reported several novel classes of nonpeptide B_2 antagonists,[19−25] represented by **2**, **3b**,**c**, and **4a** (Chart 2). Recently, a pseudopeptide[26−28] and several new nonpeptide compounds, some of which also incorporate similar skeleton to that of our FR compounds,[29−31] were described as potent B_2 receptor antagonists.

Chart 1. Representative Peptide B_2 Receptor Antagonists



In the preceding paper[32] we reported that the 4-substituent of the quinoline ring is important not only to improve aqueous solubility but also to increase the affinity for the human B_2 receptor. In this article we would like to report the SAR of the 4-alkylamino quinoline derivatives, possessing characteristic binding profiles and/or agonist/antagonist properties.

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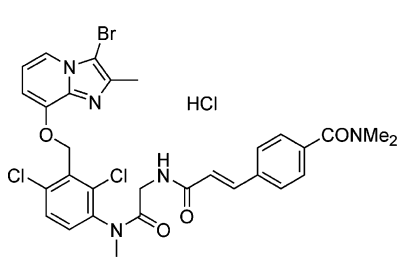
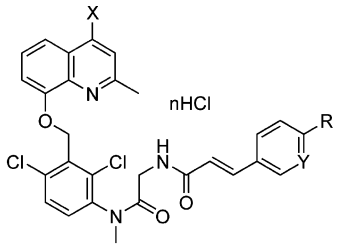
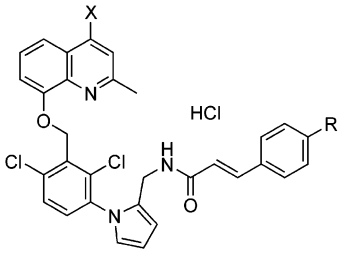
Chart 2. Representative Fujisawa Nonpeptide B_2 Antagonists (**2**, **3a**−**c** and **4a**,**b**)



2

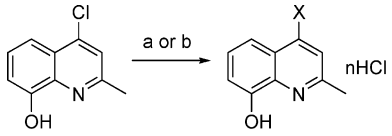


3a (FR173657): X=H, Y=N, R=NHAc, n=0
3b: X=H, Y=CH, R=CONHMe, n=1
3c: X=NMe_2, Y=CH, R=CONHMe, n=2



4a: X = H, R=CONHMe, n = 1
4b: X = imidazolyl, R=$CONMe_2$, n = 2

Scheme 1[a]



5

6a:X=1-pyrrolidinyl, n=0
6b:X=1-piperidinyl, n=0
6c:X=1-homopiperidinyl, n=0
6d:X=4-morpholinyl, n=0
6e:X=4-methyl-1-piperazinyl, n=3
6f:X=$NH(CH_2)_2OMe$, n=2
6g:X=$N[(CH_2)_2OMe]_2$, n=0
6h:X=$NH(CH_2)_2NMe_2$, n=3

[a] Reagents (a) corresponding amines, phenol, 125 °C; (b) hexamethyleneimine, *n*-Bu_4NI

Chemistry

The compounds described in this study are shown in Tables 1−4, and the methods for their synthesis are outlined in Schemes 1−4.

Preparation of the 4-(substituted)-2-methylquinolinol derivatives **6a**−**h** is shown in Scheme 1. Reaction of the 4-chloroquinolinol **5** with the corresponding amines yielded the 4-substituted-quinolinols **6a**−**h**, respectively (Scheme 1).

Preparation of the benzyl bromide **10b** is shown in Scheme 2. Condensation of **7** with (*E*)-3-(6-acetylaminopyridin-3-yl)acrylic acid[20] in the presence of 1-ethyl-3-[(dimethylamino)propyl]carbodiimide hydrochloride (WSCD·HCl) and 1-hydroxybenzotriazole (HOBt) gave the cinnamamide **8**. Removal of the silyl protecting group with tetrabutylammonium fluoride and subsequent treatment with triphenylphosphine and carbon tetrabromide in CH_2Cl_2 provided the benzyl bromide **10b**. The quinolinols **6a**−**h** and 4-(dimethylamino)-2-methyl-8-quinolinol[32] were coupled with the benzyl bromide **10a** or **10b** in the presence of K_2CO_3 to afford **11a**−**h**, **12a**, and **12b**, respectively. The 4-substituted quinoline derivatives **11a**−**h** and **12a** were treated with 10% HCl in MeOH to the corresponding hydrochlorides **13a**−**h** and **14**, respectively (Scheme 2).

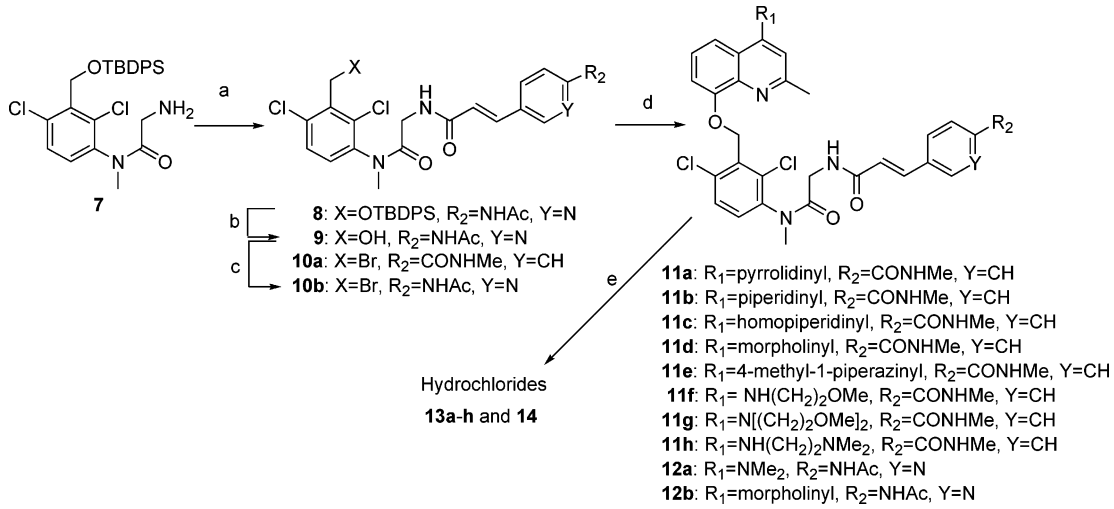
Preparation of the cinnamic acids **19** and **23** is shown in Scheme 3. Methyl 4-(acetyloxy)methyl-2-methoxybenzoate (**15**) was hydrolyzed with 1 N NaOH to afford **16**. Oxidation of the alcohol group of **16** with sulfur trioxide pyridine complex in a mixture of DMSO and CH_2Cl_2 followed by a Wittig reaction with methyl (triphenylphosphoranylidene)acetate afforded the ester **17**. The ester **17** was condensed with methylamine hydrochloride providing the amide **18**, which was hydrolyzed to give the cinnamic acid **19**.

On the other hand, acetylation of (4-amino-3-methylphenyl)methanol (**20**)[33] with acetic anhydride in MeOH gave the acetamide **21**. Oxidation of the alcohol group of **21** with sulfur trioxide pyridine complex in a mixture of DMSO and CH_2Cl_2 followed by a Perkin reaction of the aldehyde **22** with malonic acid in the presence of pyridine in EtOH afforded the cinnamic acid **23**.

Modifications of the terminal cinnamamide moiety of 4-(substituted)-2-methylquinoline derivatives are shown in Scheme 4. Compound **24** was treated with methanesulfonyl chloride followed by coupling with **6b**,**d** and 4-(dimethylamino)-2-methyl-8-quinolinol, respectively. Removal of the *N*-phthaloyl group of **25a**−**c** with hydrazine monohydrate and coupling with the (*E*)-4-(substituted)cinnamic acids afforded the corresponding cinnamamides **27b**,**c**, **28**, **29a**−**c**, **30a**−**c**, **31a**,**b**, **32**, **33a**,**c**, **37b**, and **37c**, respectively. The ethyl esters **33a** and **33c** were hydrolyzed with 1 N NaOH to give the corresponding carboxylic acids **34a** and **34c**, which were condensed with the appropriate amine hydrochloride to furnish the corresponding amides **35a**,**c** and **36**, respectively. The cinnamamide derivatives **27b**,**c**, **28**, **29a**−**c**, **30a**−**c**, **31a**,**b**, **32**, **35a**,**c**, **36**, **37b**, and **37c** were treated with 10% HCl in MeOH to afford the corresponding hydrochlorides **38b**,**c**, **39**, **40a**−**c**, **41a**−**c**, **42a**,**b**, **43**, **44a**,**c**, **45**, **46b**, and **46c**, respectively.

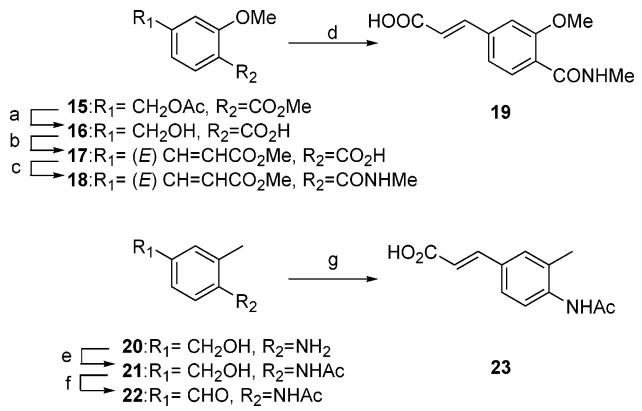
Synthesis of compound **48** is shown in Scheme 4. The amine **26a** was coupled with phenyl 3-[(4-pyridinylamino)carbonyl]phenylcarbamate[32] in the presence of Et_3N to afford the urea **47**. **47** was treated with 10% HCl in MeOH to form the corresponding hydrochloride **48**.

Scheme 2[a]



[a] Reagents (a) (*E*)-3-(6-acetylaminopyridin-3-yl)acrylic acid, WSCD·HCl, HOBt, DMF; (b) *n*-Bu_4NF, THF; (c) CBr_4, Ph_3P, CH_2Cl_2; (d) **6a**−**h**, K_2CO_3, DMF; (e) 10% HCl in MeOH.

Scheme 3[a]



[a] Reagents (a) 1 N NaOH, MeOH; (b) SO_3·Py, DMSO, CH_2Cl_2, then methyl (triphenylphosphoranylidene)acetate, THF; (c) $MeNH_2$·HCl, WSCD, HOBt, DMF; (d) 1 N NaOH, MeOH, 50 °C; (e) Ac_2O, MeOH; (f) SO_3·Py, DMSO; (g) malonic acid, pyridine, EtOH.

Biology

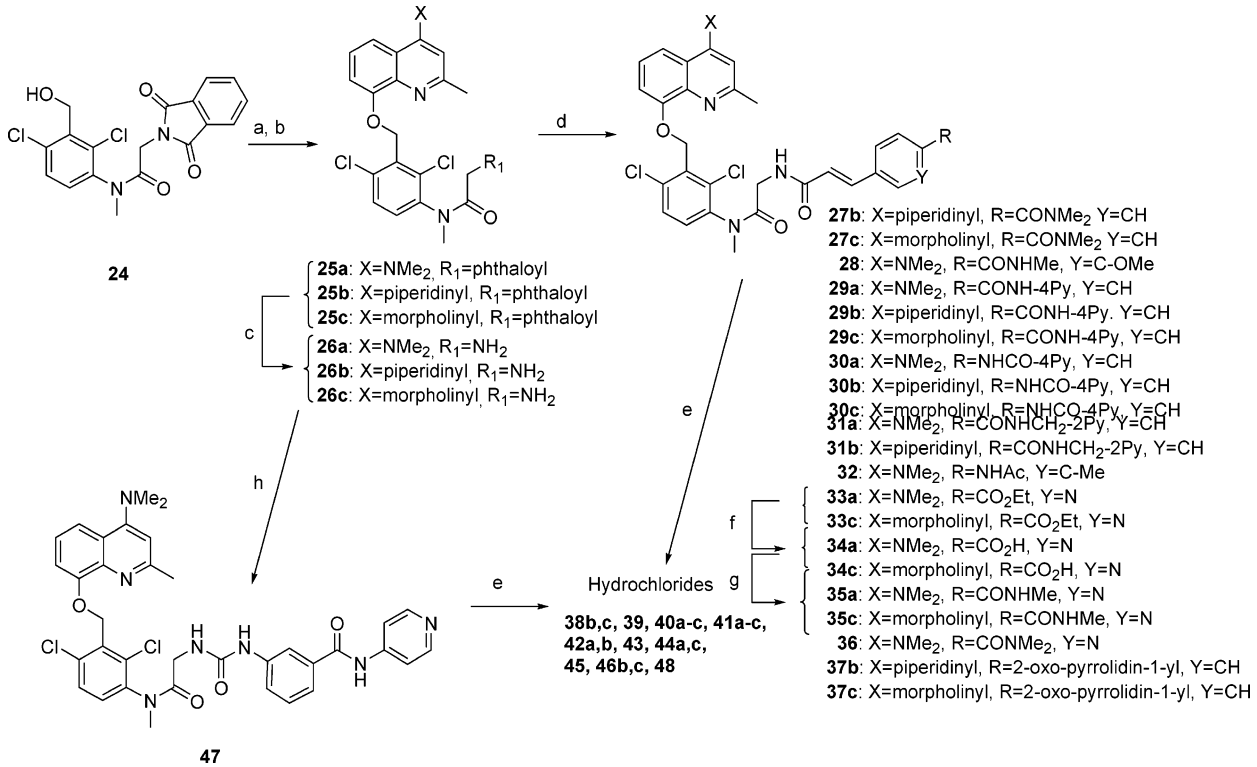
All compounds were tested for inhibition of the specific binding of [^{3}H]BK to the B_2 receptor in guinea pig ileum membrane preparations as previously reported,[19,20,23−25] and they were also evaluated for inhibition of the specific binding of [^{3}H]BK to the cloned human B_2 receptor expressed in CHO cells.[24] Selected compounds were then tested for in vivo functional antagonistic activity in inhibiting BK-induced bronchoconstriction in guinea pigs by iv administration.[19,20,23,25] Furthermore, some compounds were examined for their B_2 agonistic activity by measuring the agonist-induced IPs formation in CHO cells expressing the human B_2 receptor.

Results and Discussion

We had reported the first selective and orally active nonpeptide B_2 receptor antagonists with subnanomolar affinities for human and guinea pig B_2 receptors.[19−22,34] Successive investigation to improve aqueous solubility, aiming at development of novel therapeutic drugs which could be administered intravenously for the treatment of life-threatening inflammatory diseases, led to a new class of nonpeptide B_2 antagonists incorporating basic heteroaromatic moieties at the 4-position of the quinoline ring. One of the most potent 4-(1-imidazolyl) derivatives, **4b**, exhibited excellent subnanomolar binding affinity for the human B_2 receptor superior to that of **1**, indicating that the 4-substituent of the quinoline ring contributes not only to improvement of aqueous solubility but also to increase in affinity for the human B_2 receptor as a novel pharmacophore.[32]

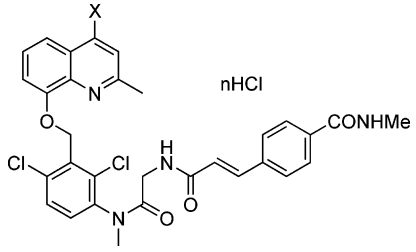
These results prompted us to carry out further detailed investigation on the SAR of this critical substituent. At the outset we intended to introduce several aliphatic amines at the 4-position of the quinoline ring in order to probe steric and electrostatic effects to B_2 binding affinities. As shown in Table 1, it was clearly indicated that the 4-substituent affected binding affinities to human and guinea pig B_2 receptors in a highly sensitive manner. 4-Dimethylamino derivative **3c** retained low nanomolar binding affinities for both B_2 receptors. On the other hand, the ring size of cyclic amines made drastic changes in the activities as follows, suggesting quite strict steric requirements. Although pyrrolidine **13a** showed much lower affinities than **3b**, piperidine **13b** recovered low nanomolar binding affinities only for the human B_2 receptor. However, homopiperidine **13c** resulted in a great loss of the activities again. Introduction of an oxygen atom (**13d**) at the 4-position of the piperidine ring of **13b** retained low nanomolar binding affinity for the human B_2 receptor and brought 5-fold increase in affinity for the guinea pig B_2 receptor. In contrast, displacement of this oxygen atom with methylamino moiety (**13e**) was not tolerated by both B_2 receptors. Morpholine and piperazine related ring-opening derivatives **13f**−**h** failed to show nanomolar binding affinities. In general, a large aliphatic amino substituent at the 4-position of the quinoline ring decreased binding affinity for the guinea pig B_2 receptor, while some of them retained low nanomolar affinities for the human B_2 receptor. We also reported in the previous paper that nitrogen containing heteroaromatic moieties afforded subnanomolar human B_2 affinity, while they resulted in several times decrease in guinea

Scheme 4[a]



[a] Reagents (a) MsCl, Et_3N, CH_2Cl_2; (b) 4-(dimethylamino)-8-hydroxy-2-methylquinoline, **6b** or **6d**, K_2CO_3, DMF; (c) $N_2H_4 \cdot H_2O$, EtOH; (d) 4-substituted cinnamic acid, WSCD·HCl, HOBt, DMF; (e) 10% HCl-MeOH, (f) 1N NaOH, EtOH, 60 °C; (g) corresponding amine·HCl, WSCD, HOBt, DMF; (h) phenyl 3-[(4-pyridinylamino)carbonyl]phenylcarbamate, Et_3N, DMF, 80 °C.

Table 1. SAR on the 4-Substituents of the Quinoline Ring



			in vitro IC_{50} (nM)	
compd	X	*n*	GP ileum[a]	cloned human B_2[b]
3b	H	1	0.51[c]	1.1[c]
3c	NMe_2	2	1.8[d]	3.2[d]
13a	1-pyrrolidinyl	2	2300	87
13b	1-piperidinyl	2	68	1.1
13c	1-homopiperidinyl	2	840	37
13d	4-morpholinyl	2	12	2.7
13e	4-Me-1-piperazinyl	3	1500	98
13f	$NH(CH_2)_2OMe$	2	49	41
13g	$N[(CH_2)_2OMe]_2$	2	560	19
13h	$NH(CH_2)_2NMe_2$	3	2300	2500

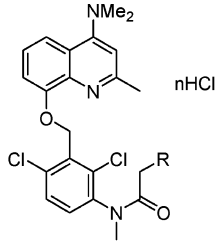
[a] Concentration required to inhibit specific binding of [^{3}H]BK (0.06 nM) to B_2 receptors in guinea pig ileum membrane preparations by 50%. Values are expressed as the average of at least three determinations, with variation in individual values of <15%. See the Experimental Section for further details. [b] Concentration required to inhibit specific binding of [^{3}H]BK (1.0 nM) to human B_2 receptors that were expressed in CHO (Chinese hamster ovary) cells by 50%. Values are expressed as the average of at least three determinations, with variation in individual values of <15%. See the Experimental Section for further details. [c] Previously published, see ref 21. [d] Previously published, see ref 32.

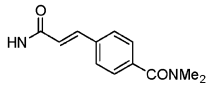
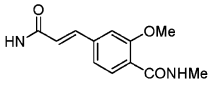
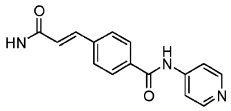
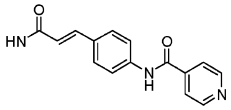
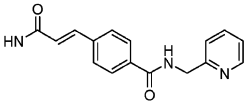
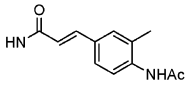
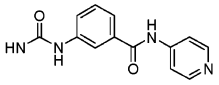
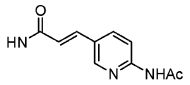
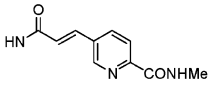
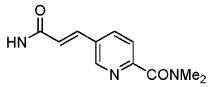
pig affinity.[32] Taken together, these results clearly indicated that human and guinea pig B_2 receptors differ in the pocket, which accommodates the critical pharmacophore at the 4-position of the quinoline ring.

Among the 4-aliphatic amino derivatives examined, 4-dimethylamino (**3c**), piperidino (**13b**), and morpholino (**13d**) derivatives, which exhibited low nanomolar binding affinities for the human B_2 receptor, were selected as lead compounds for further chemical modification. Substitution of the cinnamoyl side chain of **3c** with representative acyl moieties, identified in our previous studies,[21,32] afforded potent B_2 antagonists **14**, **38a**, **39**, **40a**, **41a**, **42a**, **43**, **44a**, **45**, and **48** which could be dissolved in 5% aqueous solution of citric acid up to the concentration of 10 mg/mL (Table 2). Compounds **14**, **38a**, **39**, **40a**, **41a**, **44a**, and **45** retained nanomolar affinities for the guinea pig B_2 receptor too, with 57 to 90-fold higher IC_{50} values compared to that of **1**. Despite such much lower affinities for the guinea pig B_2 receptor, they inhibited BK induced bronchoconstriction in guinea pig at 10 μg/kg by intravenous administration more efficaciously than **1** did at 1 μg/kg (iv). In the same way compounds **42a**, **43**, and **48** with 133- to 311-fold lower affinities for the guinea pig B_2 receptor exhibited almost complete in vivo inhibition at 100 μg/kg (iv). Their in vivo effects were as strong as that of **1** at 10 μg/kg (iv). Since one of the representative compounds, **40a** possesses comparable affinity for the human B_2 receptor with that of **1**, we are expecting that **40a** could exhibit equal or superior clinical effects to those of **1**.

Table 3 shows the results of similar attempts in 4-(4-morpholino) and 4-(1-piperidino) series. In comparison with 4-dimethylamino derivatives **14**, **40a**, **42a**, and **44a** (Table 2), similar solubility profile and larger species difference between human and guinea pig B_2 binding affinities were observed. The most potent morpholino

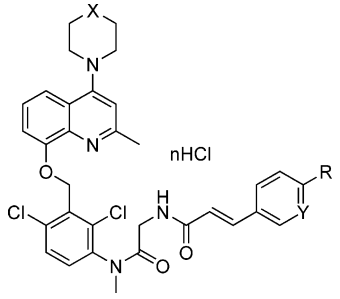
Table 2. Binding and in Vivo Antagonistic Activities of 4-Dimethylaminoquinoline Derivatives



compds	R	n	IC_{50}(nM) GP ileum[a]	IC_{50}(nM) Cloned Human B_2[b]	Inhibition (%) against BK-induced bronchoconstriction[c] 1μg/kg (iv)	10μg/kg (iv)	100μg/kg (iv)
38a		2	2.9[e]	2.1[e]	NT[d]	73.4±5.5***	NT
39		2	5.6	2.0	NT	88.7±2.5***	NT
40a		3	5.1	0.70	NT	86.3±4.4***	NT
41a		3	6.9	0.47	NT	59.0±7.6***	NT
42a		3	28	5.9	NT	NT	97.3±2.0***
43		2	21	4.8	NT	NT	94.7±4.0**
48		3	12	2.2	NT	NT	97.7±1.5***
14		3	6.3	4.2	NT	89.4±1.2***	NT
44a		3	1.2	1.6	NT	85.5±7.8**	NT
45		3	8.1	4.0	NT	89.7±2.9***	NT
1			0.09	0.49	63.4±8.9**	96.7±0.3***	NT

[a,b] See corresponding footnotes in Table 1. [c] BK (5 μg/kg) was administered intravenously to anesthetized guinea pigs, and bronchoconstriction induced by the BK administration was measured by the modified Konzett and Rösseler method[36] as previous reported. After 5 min, compounds were intravenously administrated. After 25 min, BK was administered again and bronchoconstriction was measured. Percent inhibition was calculated from the values of percent responses of drug-treated and controlled groups ($n = 3-4$). The results are expressed as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs control (Student's *t*-test). See the Experimental Section for further details. [d] NT, not tested. [e] Previously published, see ref 32.

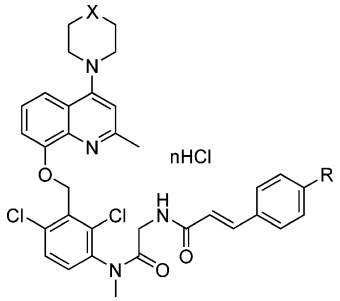
Table 3. Binding and in Vivo Antagonistic Activities of Morpholinyl- and Piperidinylquinoline Derivatives



					IC_{50}(nM)		inhibition (%) against BK-induced bronchoconstriction[c]	
compds	X	Y	R	*n*	GP ileum[a]	cloned human B_2[b]	10 μg/kg (iv)	100 μg/kg (iv)
40c	O	CH	CONH-4Py	3	37	0.64	46.2 ± 9.3*	91.8 ± 2.1***
12b	O	N	NHAc	0	65	1.8	NT[d]	47.8 ± 9.3***
44c	O	N	CONHMe	3	85	1.1	NT	72.3 ± 9.9**
42b	CH	CH	$CONHCH_2$-2Py	3	40	2.5	NT	72.6 ± 8.0*
1					0.09	0.49	96.7 ± 0.3***	NT

[a,b,c,d] See corresponding footnotes in Table 1 and Table 2.

Table 4. Binding and B_2 Agonistic Activities of Morpholinyl- and Piperidinylquinoline Derivatives



				IC_{50} (nM)		relative agonistic activity (%) in IP formation compared to BK (10 nM)[c]	
compds	R	X	*n*	GP ileum[a]	cloned human B_2[b]	1 μM	10 μM
13b	CONHMe	CH_2	2	68	1.1	4.4 ± 0.9	4.2 ± 0.6
13d	CONHMe	O	2	12	2.7	0.9 ± 2.7	−1.9 ± 2.0
38b	$CONMe_2$	CH_2	2	30	0.76	17.2 ± 0.9	21.8 ± 1.4
38c	$CONMe_2$	O	2	43	0.88	5.9 ± 0.5	7.4 ± 0.3
40b	CONH-4Py	CH_2	3	240	0.52	5.7 ± 2.2	12.7 ± 1.2
40c	CONH-4Py	O	3	37	0.64	0.1 ± 0.5	−0.3 ± 0.2
41b	NHCO-4Py	CH_2	3	75	1.6	5.6 ± 0.6	5.3 ± 0.7
41c	NHCO-4Py	O	3	29	1.0	−0.10 ± 0.4	0.9 ± 0.2
46b	2-oxopyrrolidin-1-yl	CH_2	2	28	1.2	14.8 ± 0.4	18.7 ± 2.1
46c	2-oxopyrrolidin-1-yl	O	2	13	1.8	6.0 ± 0.2	9.2 ± 0.6

[a,b] See corresponding footnotes in Table 1. [c] Inositol phosphates (IPs) production was measured essentially as described previously.[24] See the Experimental Section for further details.

derivative **40c** significantly inhibited BK induced bronchoconstriction at 10 μg/kg (iv) despite 411-fold lower affinity for the guinea pig B_2 receptor compared to that of **1**. A piperidino derivative **42b** with similar affinity for the guinea pig B_2 receptor also showed significant inhibition at 100 μg/kg (iv).

Our representative nonpeptide B_2 antagonists previously reported[20,21,32] including compounds **2**, **3a**−**c**, **4a**, and **4b** had been confirmed not to affect the formation of the second messengers, IPs in CHO cells expressing the cloned human B_2 receptor. Namely, they had been proven not to have any agonistic or inverse agonistic properties. The B_2 ligands described above in this paper except for **13b** also showed pure antagonistic profiles. However, some of the 4-(4-morpholino) and 4-(1-piperidino) derivatives **13b**, **38b**,**c**, **40b**, **41b**, and **46b**,**c** (Table 4) were revealed to increase IPs formation significantly at the concentrations of 1 and 10 μM compared with the control IPs formation determined in the absence of the compounds (by Student's *t*-test, at 1 μM **40b**: $P < 0.05$, **13b**: $P < 0.01$, **38b**,**c**, **41b**, **46b**,**c**: $P < 0.001$; at 10 μM **40b**: $P < 0.01$, **13b**, **38b**,**c**, **41b**, **46b**,**c**: $P < 0.001$). The B_2 agonistic activity was expressed as a percentage relative to the maximum effect of BK exerted at 10 nM concentration. Generally, piperidino derivatives were more efficacious than morpholino congeners. The 4-(4-morpholino) and 4-(1-piperidino) moieties seem to be critical for B_2 agonistic activity, while the acyl side chains of each series also make contribution to modify the efficacy. Among the compounds tested, the best combination to produce agonistic activity was that of 4-(1-piperidino) moiety and 4-(dimethylcarbamoyl)cin-

namide side chain in compound **38b**. Although the potency and efficacy of these partial agonists were much lower than those of BK, their B_2 agonistic activity was significant enough to afford a new insight into our study on nonpeptide B_2 receptor ligands. Thus we identified screening leads for nonpeptide B_2 agonists.

Conclusion

Various aliphatic amines were introduced to the 4-position of the quinoline ring. Their SAR clearly indicated that the 4-substituent affected binding affinities to both human and guinea pig B_2 receptors in a highly sensitive manner. Among them 4-dimethylamino (**3c**), piperidino (**13b**), and morpholino (**13d**) derivatives exhibited low nanomolar binding affinities for the human B_2 receptor. Substitution of their acyl side chains with representative ones, identified in our previous studies, afforded potent and pure antagonists for iv use as well as unique partial agonists for the human B_2 receptor. Representative 4-dimethylamino derivative **40a** inhibited BK induced bronchoconstriction in vivo at 10 μg/kg by intravenous administration more efficaciously than **1** did at 1 μg/kg (iv), despite 57-fold lower binding affinity for the guinea pig B_2 receptor compared to that of **1**. Since **40a** possesses comparable affinity for the human B_2 receptor with that of **1**, it is expected that **40a** could exhibit equal or superior clinical effects to those of **1**. On the other hand several 4-(4-morpholino) and 4-(1-piperidino) derivatives were revealed to increase IPs formation by themselves in CHO cells expressing the cloned human B_2 receptor. They were the first examples with agonistic activities in our nonpeptide B_2 ligands. Although the potency and efficacy of these partial agonists were much lower than those of BK, we identified them as screening leads for nonpeptide B_2 agonists. Extensive investigation for nonpeptide B_2 full agonists and their in vivo pharmacology will be discussed in due course.

Experimental Section

Chemistry. Melting points were determined on a Mel-Temp instrument (Mitamura Riken Kogyo, Japan) and are uncorrected. Proton NMR spectra were recorded at 200 or 300 MHz with a Bruker AM200 or a Varian Gemini 300 spectrometer, and chemical shifts are expressed in δ (ppm) with TMS as internal standard. The peak patterns are shown as the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. The mass spectra (MS) were recorded with a VG (Fisons) ZAB−SE (FAB) or Micromass Platform (ESI) system. Elemental analyses were performed on a Perkin-Elmer 2400 CHN analyzer. Analytical results were within ±0.4% of the theoretical values unless otherwise noted. Silica gel $60F_{254}$ precoated plates on glass from Merck KGaA or amonopropyl silicagel (APS) precoated NH plates from Fuji Silysia Chemical Ltd. were used for thin-layer chromatography. Silica gel chromatographies were performed with Kieselgel 60 (230−400 mesh) (Merck KGaA, Darmstadt, Germany) or NH-DM 1020 (Fuji Silysia Chemical Ltd., Japan). Yields were not optimized.

2-Methyl-4-(1-pyrrolidinyl)-8-quinolinol (6a). A mixture of 4-chloro-2-methyl-8-quinolinol (**5**)[32] (300 mg, 1.55 mmol), pyrrolidine (165 mg, 2.32 mmol), and phenol (292 mg, 3.1 mmol) was stirred at 125 °C for 5 h. Pyrrolidine (165 mg, 2.32 mmol) was added to the reaction mixture, and the mixture was stirred at 125 °C for additional 5 h. After cooling, the resulting residue was crystallized from acetone. The solid was dissolved in $CHCl_3$ and the solution washed with a mixture of aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated in vacuo. The resulting residue was crystallized from Et_2O to afford **6a** (109 mg, 30.8%) as pale-brown crystals: mp 135−137 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.99−2.10 (m, 4H), 2.56 (s, 3H), 3.65−3.76 (m, 4H), 6.32 (s, 1H), 7.03 (d, J = 8 Hz, 1H), 7.16 (dd, J = 8, 8 Hz, 1H), 7.65 (d, J = 8 Hz, 1H); MS (ESI) m/z 229 (M + 1); Anal. ($C_{14}H_{16}N_2O$) C, H, N.

Compounds **6b**, **6d**−**g**, and **6h** were prepared following the procedure described above for **6a**.

4-(1-Azepanyl)-2-methyl-8-quinolinol (6c). A mixture of **5** (200 mg, 1.03 mmol), tetrabutylammonium iodide (10 mg), and hexamethyleneimine (1.02 g, 10.3 mmol) was refluxed for 2 h. After cooling, the resulting residue was dissolved in $CHCl_3$ and the solution washed with aqueous $NaHCO_3$, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by flash silica gel chromatography (NH-DM 1020, $CHCl_3$) to afford **6c** (259 mg, 97.8%) as a brown oil: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.70−1.80 (m, 4H), 1.87−1.99 (m, 4H), 2.59 (s, 3H), 3.49−3.58 (m, 4H), 6.63 (s, 1H), 7.03 (d, J = 8 Hz, 1H), 7.21 (dd, J = 8, 8 Hz, 1H), 7.46 (d, J = 8 Hz, 1H); MS (ESI) m/z 257 (M + 1); Anal. ($C_{16}H_{20}N_2O$) C, H, N.

(2*E*)-3-[6-(Acetylamino)-3-pyridinyl]-*N*-{2-[3-({[*tert*-butyl(diphenyl)silyl]oxy}methyl)-2,4-dichloromethylanilino]-2-oxoethyl}-2-propenamide (8). To a solution of **7** (1.20 g, 2.39 mmol) in dry DMF (12 mL) were added (*E*)-3-(6-acetylaminopyridin-3-yl)acrylic acid (501 mg, 2.44 mmol), 1-ethoxy-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (WSCD•HCl; 550 mg, 2.87 mmol), and 1-hydroxybenzotriazole (HOBt; 420 mg, 3.11 mmol) at ambient temperature. After 3 h, this mixture was partitioned between CH_2Cl_2 and water. The organic layer was washed with saturated aqueous $NaHCO_3$, water, and brine, dried over $MgSO_4$, and evaporated in vacuo. The resulting residue was crystallized from EtOAc to afford **8** (1.24 g, 75.4%) as pale-yellow crystals: mp 194−196 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.06 (s, 9H), 2.22 (s, 3H), 3.23 (s, 3H), 3.57 (dd, J = 17, 4 Hz, 1H), 3.94 (dd, J = 17, 5 Hz, 1H), 4.92 (d, J = 10 Hz, 1H), 4.98 (d, J = 10 Hz, 1H), 6.44 (d, J = 15 Hz, 1H), 6.63 (br s, 1H), 7.22 (d, J = 8 Hz, 1H), 7.35−7.48 (m, 6H), 7.52 (d, J = 15 Hz, 1H), 7.70−7.77 (m, 4H), 7.83 (dd, J = 8, 3 Hz, 1H), 8.05 (br s, 1H), 8.22 (d, J = 8 Hz, 1H), 8.36 (d, J = 3 Hz, 1H); Anal. ($C_{36}H_{38}Cl_2N_4O_4Si$) C, H, N.

(2*E*)-3-[6-(Acetylamino)-3-pyridinyl]-*N*-{2-[2,4-dichloro-3-(hydroxymethyl)methylanilino]-2-oxoethyl}-2-propenamide (9). To a suspension of **8** (940 mg, 1.36 mmol) in THF (9.4 mL) was added 1 M tetrabutylammonium fluoride in THF (2.04 mL) at ambient temperature. After 1 h, the mixture was partitioned between CH_2Cl_2 and water. The organic layer was washed with 1 N HCl, water, saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was crystallized from CH_3CN to afford **9** (569 mg, 92.4%) as colorless crystals: mp 207−209 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.10 (s, 3H), 3.10 (s, 3H), 3.47 (dd, J = 17, 4 Hz, 1H), 3.76 (dd, J = 17, 5 Hz, 1H), 4.74 (d, J = 5 Hz, 1H), 5.35 (br s, 1H), 6.79 (d, J = 15 Hz, 1H), 7.37 (d, J = 15 Hz, 1H), 7.61 (d, J = 8 Hz, 1H), 7.65 (d, J = 8 Hz, 1H), 7.98 (dd, J = 8, 3 Hz, 1H), 8.11 (d, J = 8 Hz, 1H), 8.21 (t, J = 5 Hz, 1H), 8.47 (s, 1H); Anal. ($C_{20}H_{20}Cl_2N_4O_4$) C, H, N.

(2*E*)-3-[6-(Acetylamino)-3-pyridinyl]-*N*-{2-[3-(bromomethyl)-2,4-dichloromethylanilino]-2-oxoethyl}-2-propenamide (10b). To a solution of **9** (555 mg, 1.23 mmol) in CH_2Cl_2 (10 mL) were added triphenylphosphine (387 mg, 1.48 mmol) and carbon tetrabromide (612 mg, 1.84 mmol) in an ice−water bath. After 10 min, the reaction mixture was stirred at ambient temperature for 3 h. To the mixture were added triphenylphosphine (96.8 mg, 0.369 mmol) and carbon tetrabromide (163 mg, 0.49 mmol), and the reaction mixture was stirred for another 2 h. The reaction mixture was washed with saturated aqueous $NaHCO_3$, water, and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by flash silica gel chromatography (CH_2Cl_2/MeOH, 20:1) to afford **10b** (400 mg, 63.3%) as pale-yellow crystals: mp 222−223 °C; ^{1}H NMR (300 MHz, $CDCl_3$–CD_3OD): δ 2.22 (s, 3H), 3.27 (s, 3H), 3.60 (dd, J = 17, 3 Hz, 1H), 3.94 (dd, J = 17, 3 Hz, 1H), 4.78 (s, 2H), 6.49 (d, J = 15 Hz, 1H), 7.31 (d, J = 8 Hz, 1H), 7.49 (d, J = 8 Hz, 1H), 7.51 (d, J = 15 Hz, 1H), 7.88 (dd, J =

8, 3 Hz, 1H), 8.23 (br d, J = 8 Hz, 1H), 8.33 (d, J = 3 Hz, 1H); Anal. ($C_{20}H_{19}BrCl_2N_4O_3$) C, H, N.

4-[(1*E*)-3-({2-[2,4-Dichloro(methyl)-3-({[2-methyl-4-(1-pyrrolidinyl)-8-quinolinyl]oxy}methyl)anilino]-2-oxoethyl}amino)-3-oxo-1-propenyl]-*N*-methylbenzamide (11a). To a mixture of compound **10a** (80.9 mg, 0.158 mmol) and **6a** (36.0 mg, 0.158 mmol) in dry DMF (1.0 mL) was added K_2CO_3 (65.5 mg, 0.474 mmol) at ambient temperature, and the mixture was stirred at same temperature for 5 h. The reaction mixture was poured into water and extracted with CH_2Cl_2 twice. The extracts were washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by preparative thin-layer chromatography ($CHCl_3$/MeOH, 10:1) to afford **11a** (85.0 mg, 81.6%) as a colorless amorphous solid: mp 169−172 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.98−2.06 (m, 4H), 2.54 (s, 3H), 2.99 (d, J = 5 Hz, 3H), 3.24 (s, 3H), 3.59−3.72 (m, 5H), 3.93 (dd, J = 17, 5 Hz, 1H), 5.56 (d, J = 10 Hz, 1H), 5.60 (d, J = 10 Hz, 1H), 6.36−6.41 (m, 2H), 6.52 (d, J = 15 Hz, 1H), 6.85 (br s, 1H), 7.11−7.30 (m, 3H), 7.41−7.50 (m, 3H), 7.55 (d, J = 15 Hz, 1H), 7.71 (br d, J = 8 Hz, 2H), 7.84 (br d, J = 8 Hz, 2H); Anal. ($C_{35}H_{35}Cl_2N_5O_4$) C, H, N.

Compounds **11b**−**h**, **12a**, and **12b** were prepared following the procedure described above for **11a**.

4-[(1*E*)-3-({2-[2,4-Dichloro(methyl)-3-({[2-methyl-4-(1-pyrrolidinyl)-8-quinolinyl]oxy}methyl)anilino]-2-oxoethyl}amino)-3-oxo-1-propenyl]-*N*-methylbenzamide Dihydrochloride (13a). To a solution of **11a** (60.0 mg, 0.091 mmol) in MeOH (0.9 mL) was added 10% HCl in MeOH (0.5 mL) at ambient temperature. After 5 min, the solution was evaporated in vacuo. The resulting residue was washed with Et_2O to afford **13a** (52.0 mg, 78.1%) as colorless crystals: mp 203−206 °C; ^{1}H NMR (300 MHz, $CDCl_3$−CD_3OD): δ 2.14−2.26 (m, 4H), 2.67 (s, 3H), 2.99 (s, 3H), 3.29 (s, 3H), 3.87 (d, J = 17 Hz, 1H), 3.89−4.08 (m, 4H), 4.13 (d, J = 17 Hz, 1H), 5.48 (d, J = 10 Hz, 1H), 5.65 (d, J = 10 Hz, 1H), 6.51 (s, 1H), 6.62 (d, J = 15 Hz, 1H), 7.33−7.64 (m, 7H), 7.81 (d, J = 8 Hz, 2H), 8.02 (d, J = 8 Hz, 1H); Anal. ($C_{35}H_{35}Cl_2N_5O_4$·2HCl) C, H, N.

Compounds **13b**−**h**, **14**, **38b,c**, **39**, **40a**−**c**, **41a**−**c**, **42a**,**b**, **43**, **44a**,**c**, **45**, **46b**,**c**, and **48** were prepared following the procedure described above for **13a**.

4-(Hydroxymethyl)-2-methoxybenzoic Acid (16). To a solution of methyl 4-[(acetyloxy)methyl]-2-methoxybenzoate (**15**) (8.82 g, 37.0 mmol) in MeOH (200 mL) was added 1 N NaOH (75 mL) at ambient temperature. The reaction mixture was stirred at same temperature for 3 h. The solvent was evaporated, and the residue was dissolved in water. The water layer was washed with Et_2O and adjusted to pH 4 with concentrated HCl. The mixture was concentrated in vacuo and EtOH was added to the residue. The precipitates were filtered off. The filtrate was evaporated and the residue dried in a vacuum to afford **16** (4.62 g, 78.5%) as a pale yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.81 (s, 3H), 4.52 (s, 2H), 5.30 (br s, 1H), 6.92 (d, J = 8 Hz, 1H), 7.08 (s, 1H), 7.61 (d, J = 8 Hz, 1H); MS (ESI) m/z 183 (M + 1); Anal. ($C_9H_{10}O_4$) C, H.

2-Methoxy-4-[(1*E*)-3-methoxy-3-oxo-1-propenyl]benzoic Acid (17). To a mixture of **16** (1.5 g, 8.23 mmol) and Et_3N (5.00 g, 49.4 mmol) in a mixture of CH_2Cl_2 (7.5 mL) and DMSO (7.5 mL) was added sulfur trioxide pyridine complex (3.93 g, 24.7 mmol) portionwise in water bath. The mixture was stirred at ambient temperature for 2 h. The reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. To a solution of the residue in THF (10 mL) was added methyl (triphenylphosphoranylidene)-acetate (3.30 g, 9.88 mmol) at ambient temperature under nitrogen. The reaction mixture was stirred at same temperature for 1 h. The reaction mixture was concentrated in vacuo and partitioned between EtOAc and saturated aqueous $NaHCO_3$. The water layer was adjusted to pH 4 with 1 N HCl and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was washed with hot IPE to afford **17** (1.21 g, 62.1%) to a solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (s, 3H), 4.12 (s, 3H), 6.53 (d, J = 15 Hz, 1H), 7.13 (s, 1H), 7.30 (d, J = 8 Hz, 1H), 7.68 (d, J = 15 Hz, 1H), 8.20 (d, J = 8 Hz, 1H); MS (ESI) m/z 237 (M + 1); Anal. ($C_{12}H_{12}O_5$) C, H.

Methyl (2*E*)-3-{3-Methoxy-4-[(methylamino)carbonyl]-phenyl}-2-propenoate (18). To a solution of **17** (500 mg, 2.12 mmol) in dry DMF (20 mL) were added methylamine hydrochloride (153 mg, 2.33 mmol), WSCD (487 mg, 2.54 mmol), and HOBt (372 mg, 2.75 mmol) at ambient temperature. After 5 h, this mixture was partitioned between EtOAc and saturated aqueous sodium bicarbonate. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was crystallized from IPE to afford **18** (448 mg, 84.9%) as a colorless amorphous solid: mp 127−128 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01 (d, J = 5 Hz, 3H), 3.82 (s, 3H), 4.00 (s, 3H), 6.49 (d, J = 15 Hz, 1H), 7.08 (s, 1H), 7.25 (d, J = 8 Hz, 1H), 7.67 (d, J = 15 Hz, 1H), 7.78 (br s, 1H), 8.23 (d, J = 8 Hz, 1H); MS (ESI) m/z 250 (M + 1); Anal. ($C_{13}H_{15}NO_4$) C, H, N.

Compounds **35a**,**c** and **36** were prepared following the procedure described above for **18**.

(2*E*)-3-[3-Methoxy-4-[(methylamino)carbonyl]phenyl]-2-propenoic Acid (19). To a solution of **18** (300 mg, 1.20 mmol) in MeOH (6 mL) was added 1 N NaOH (1.5 mL) at ambient temperature. The reaction mixture was stirred at 50 °C for 5 h. The solvent was evaporated in vacuo, and the residue was dissolved in water. The water layer was washed with Et_2O and adjusted to pH 4 with 1 N HCl. The solid that precipitated was collected by vacuum filtration and washed with water to afford **19** (250 mg, 88.3%) as a pale-yellow amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.79 (d, J = 5 Hz, 3H), 3.91 (s, 3H), 6.66 (d, J = 15 Hz, 1H), 7.31 (d, J = 8 Hz, 1H), 7.43 (s, 1H), 7.60 (d, J = 15 Hz, 1H), 7.73 (d, J = 8 Hz, 1H), 8.16 (q, J = 5 Hz, 1H); MS (ESI) m/z 236 (M + 1); Anal. ($C_{12}H_{13}NO_4$) C, H, N.

***N*-[4-(Hydroxymethyl)-2-methylphenyl]acetamide (21).** To a solution of (4-amino-3-methylphenyl)methanol (**20**) (1.77 g, 12.9 mmol) in MeOH (18 mL) was added Ac_2O (3.7 mL, 39.2 mmol) at ambient temperature, and the reaction mixture was stirred at same temperature for 2 h. The reaction mixture was evaporated in vacuo, and the residue was dissolved in EtOAc. The solution was concentrated in vacuo, and the residue was washed with Et_2O to afford **21** (1.90 g, 82.2%) as a solid: mp 103−104 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.03 (s, 3H), 2.18 (s, 3H), 4.42 (d, J = 6 Hz, 2H), 5.09 (t, J = 6 Hz, 1H), 7.07 (d, J = 8 Hz, 1H), 7.13 (s, 1H), 7.31 (d, J = 8 Hz, 1H), 9.24 (s, 1H); Anal. ($C_{10}H_{13}NO_2$) C, H, N.

***N*-(4-Formyl-2-methylphenyl)acetamide (22).** To a mixture of **21** (1.90 g, 10.6 mmol) and triethylamine (7.4 mL, 53.1 mmol) in DMSO (9.5 mL) was added sulfur trioxide pyridine complex (3.71 g, 23.3 mmol) portionwise in water bath. The mixture was stirred at ambient temperature for 2 h. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was crystallized from IPE to afford **22** (1.25 g, 66.2%) as colorless crystals: mp 110−112 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.26 (s, 3H), 2.33 (s, 3H), 7.16 (br s, 1H), 7.70−7.78 (m, 2H), 8.30 (br s, 1H), 9.91 (s, 1H); Anal. ($C_{10}H_{11}NO_2$) C, H, N.

(2*E*)-3-[4-(Acetylamino)-3-methylphenyl]-2-propenoic Acid (23). A mixture of **22** (1.23 g, 6.94 mmol) and malonic acid (795 mg, 7.64 mmol) in dry pyridine (549 mg, 6.94 mmol) and EtOH (3.4 mL) was refluxed for 3 h. After the mixture was cooled, the solid that precipitated was collected by vacuum filtration to afford **23** (1.08 g, 71.0%) as a pale yellow amorphous solid: mp 262−263 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.09 (s, 3H), 2.23 (s, 3H), 6.43 (d, J = 16 Hz, 1H), 7.43−7.61 (m, 4H), 9.33 (s, 1H); Anal. ($C_{12}H_{13}NO_3$) C, H, N.

***N*-[2,4-Dichloro-3-({[4-(dimethylamino)-2-methyl-8-quinolinyl]oxy}methyl)phenyl]-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-methylacetamide (25a). Step 1**. To a solution of **24** (2.00 g, 5.09 mmol) and Et_3N (772 mg, 7.63 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise methanesulfonyl chloride (641 g, 5.60 mmol) in an ice−water bath

under nitrogen. After 30 min, the reaction mixture was washed with water, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and evaporated in vacuo to afford the methanesulfonate intermediate (2.40 g, ~100%) as a pale-yellow oil.

Step 2. To a mixture of 4-(dimethylamino)-8-hydroxy-2-methylquinoline[33] (1.02 g, 5.04 mmol) and K_2CO_3 in dry DMF (25 mL) was added dropwise a solution of methanesulfonate intermediate (2.40 g, 5.09 mmol) in dry DMF (25 mL), and the mixture was stirred at ambient temperature for 1 day. The reaction mixture was poured into water and extracted with $CHCl_3$ twice. The extracts were washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was crystallized from a mixture of isopropyl alcohol and water to afford **25a** (2.36 g, 81.0%) as colorless crystals: mp 137−139 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.66 (s, 3H), 2.96 (s, 6H), 3.21 (s, 3H), 4.07 (s, 2H), 5.63 (d, J = 10 Hz, 1H), 5.71 (d, J = 10 Hz, 1H), 6.69 (s, 1H), 7.20 (d, J = 8 Hz, 1H), 7.30 (d, J = 8 Hz, 1H), 7.46 (d, J = 8 Hz, 1H), 7.53 (d, J = 8 Hz, 1H), 7.65−7.75 (m, 3H), 7.82−7.90 (m, 2H); Anal. ($C_{30}H_{26}Cl_2N_4O_4$) C, H, N.

Compounds **25b** and **25c** were prepared following the procedure described above for **25a.**

2-Amino-*N*-[2,4-dichloro-3-({[4-(dimethylamino)-2-methyl-8-quinolinyl]oxy}methyl)phenyl]-*N*-methylacetamide (26a). To a suspension of **25a** (2.44 g, 4.23 mmol) in EtOH (25 mL) was added hydrazine monohydrate (423 mg, 8.45 mmol) at ambient temperature, and the mixture was refluxed for 1 h. After the reaction mixture was cooled, the precipitates formed were filtered off. The filtrate was evaporated in vacuo, to the residue was added $CHCl_3$ (20 mL), and precipitates were filtered off. The filtrate was evaporated in vacuo, and the residue was purified by silica gel chromatography (NH-DM1020, $CHCl_3$) to afford **26a** (1.40 g, 74.1%) as a colorless amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.66 (s, 3H), 2.91−3.13 (m, 8H), 3.21 (s, 3H), 5.61 (s, 2H), 6.70 (s, 1H), 7.12−7.36 (m, 3H), 7.45 (d, J = 8 Hz, 1H), 7.70 (d, J = 8 Hz, 1H); MS (ESI) m/z 447 (M + 1); Anal. ($C_{22}H_{24}Cl_2N_4O_2$) C, H, N.

Compounds **26b** and **26c** were prepared following the procedure described above for **26a.**

4-[(1*E*)-3-({2-[2,4-Dichloro(methyl)-3-({[2-methyl-4-(1-piperidinyl)-8-quinolinyl]oxy}methyl)anilino]-2-oxoethyl}amino)-3-oxo-1-propenyl]-*N*,*N*-dimethylbenzamide (27b). To a solution of **26b** (30 mg, 0.062 mmol) in dry DMF (1 mL) were added (*E*)-4-(*N*,*N*-dimethylcarbamoyl)-cinnamic acid[19] (14.8 mg, 0.068 mmol), WSCD•HCl (14.2 mg, 0.074 mmol), and HOBt (10.8 mg, 0.08 mmol) at ambient temperature. After 3 h, this mixture was partitioned between EtOAc and saturated aqueous $NaHCO_3$. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by preparative thin-layer chromatography ($CHCl_3$/MeOH, 10:1) to afford **27b** (42 mg, 99.1%) as a pale-yellow amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60−1.75 (m, 2H), 1.79−1.90 (m, 4H), 2.68 (br s, 3H), 2.98 (br s, 3H), 3.06−3.29 (m, 10H), 3.70 (br d, J = 17 Hz, 1H), 3.97 (br d, J = 17 Hz, 1H), 5.60 (br s, 2H), 6.52 (br d, J = 15 Hz, 1H), 6.71 (s, 1H), 7.20 (br d, J = 8 Hz, 1H), 7.27−7.60 (m, 9H), 7.62 (br d, J = 8 Hz, 1H); MS (ESI) m/z 688 (M + 1); Anal. ($C_{37}H_{39}Cl_2N_5O_4$) C, H, N.

Compounds **27c**, **28**, **29a**−**c**, **30a**−**c, 31a**,**b**, **32**, **33a**,**c**, **37b**, and **37c** were prepared following the procedure described above for **27b**.

5-[(1*E*)-3-({2-[2,4-Dichloro-3-({[4-(dimethylamino)-2-methyl-8-quinolinyl]oxy}methyl)methylanilino]-2-oxoethyl}amino)-3-oxo-1-propenyl]-2-pyridinecarboxylic acid (34a). A suspension of **33a** (948 mg, 1.46 mmol) in EtOH (8 mL) containing 1N NaOH (1.6 mL) was heated at 60 °C for 2 h. Upon cooling, the reaction mixture was adjusted to pH 7 with 1 N HCl and concentrated in vacuo. The residue was partitioned between a mixture of $CHCl_3$ and MeOH (5:1) and brine. The organic layer was dried over $MgSO_4$ and evaporated in vacuo. The residue was triturated with CH_3CN to afford **34a** (771 mg, 85.0%) as a pale-yellow amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.60 (s, 3H), 3.13 (s, 3H), 3.20−3.42 (m, 6H), 3.58 (br dd, J = 17, 4 Hz, 1H), 3.90 (br dd, J = 17, 5 Hz, 1H), 5.51 (d, J = 10 Hz, 1H), 5.58 (d, J = 10 Hz, 1H), 6.90 (br s, 1H), 7.01 (d, J = 15 Hz, 1H), 7.44−7.93 (m, 6H), 8.07 (d, J = 8 Hz, 1H), 8.14 (br d, J = 8 Hz, 1H), 8.45 (br t, J = 5 Hz, 1H), 8.88 (br s, 1H); MS (ESI) m/z 622 (M + 1); Anal. ($C_{31}H_{29}Cl_2N_5O_5$) C, H, N.

Compound **34c** was prepared following the procedure described above for **34a**.

3-{[({2-[2,4-Dichloro-3-({[4-(dimethylamino)-2-methyl-8-quinolinyl]oxy}methyl)methylanilino]-2-oxoethyl}amino)carbonyl]amino}-*N*-(4-pyridinyl)benzamide (47). A mixture of **26a** (80 mg, 0.179 mmol), phenyl 3-[(4-pyridinylamino)carbonyl]phenylcarbamate (62.6 mg, 0.188 mmol), and Et_3N (109 mg, 1.07 mmol) in dry DMF (1.5 mL) was stirred at ambient temperture for 5 h. The reaction mixture was poured into water and extracted with $CHCl_3$. The organic layer was washed with water three times, dried over $MgSO_4$, and evaporated in vacuo. The residue was purified by preparative thin-layer chromatography ($CHCl_3$/MeOH/NH_4OH, 10:1:0.1) to afford **47** (61.0 mg, 49.7%) as a pale-yellow amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.50 (s, 3H), 3.01 (s, 6H), 3.19 (s, 3H), 3.87−3.98 (m, 2H), 5.35−5.49 (m, 2H), 6.32 (br peak, 1H), 6.65 (s, 1H), 7.03 (dd, J = 8, 8 Hz, 1H), 7.10−7.20 (m, 2H), 7.28−7.42 (m, 4H), 7.59−7.68 (m, 1H), 7.70 (d, J = 8 Hz, 1H), 7.78 (d, J = 6 Hz, 1H), 8.40−8.52 (m, 2H), 8.69 (br s, 1H), 9.47 (br peak, 1H); MS (ESI) m/z 686 (M + 1); Anal. ($C_{35}H_{33}Cl_2N_7O_4$) C, H, N.

Biological Methods. Receptor Binding: Guinea Pig Ileum. The specific binding of [^{3}H]BK (a high affinity B_2 ligand) was assayed according to the method previously described[35] with minor modifications. Male Hartley guinea pigs (from Charles River Japan, Inc.) were killed by exsanguination under anesthesia. The ilea were removed and homogenized in ice-cooled buffer (50 mM sodium (trimethylamino)ethanesulfonate (TES) and 1 mM 1,10-phenanthroline, pH 6.8) with Polytron. The homogenate was centrifuged to remove cellular debris (1000*g*, 20 min, 4 °C), and the supernatant was centrifuged (100 000*g*, 60 min, 4 °C). The pellet was then resuspended in ice-cooled binding buffer (50 mM TES, 1 mM 1,10-phenanthroline, 140 μg/mL bacitracin, 1 mM dithiothreitol, 1 μM captopril, and 0.1% bovine serum albumin (BSA), pH 6.8) and was stored at −80 °C until use.

In the binding assay, the membranes (0.2 mg of protein/mL) were incubated with 0.06 nM of [^{3}H]BK and varying concentrations of test compounds or unlabeled BK at room temperature for 60 min. Receptor-bound [^{3}H]BK was harvested by filtration through Whatman GF/B glass fiber filters under reduced pressure, and the filter was washed five times with 300 μL of ice-cooled buffer (50 mM Tris-HCl). The radioactivity retained on the washed filter was measured with a liquid scintillation counter. Specific binding was calculated by subtracting the nonspecific binding (determined in the presence of 1 μM unlabeled BK) from total binding. All experiments were carried out three times.

Cloned Human B_2 Receptors Expressed in CHO Cells. CHO (dhfr$^-$) cells that were transfected with and stably expressed with human B_2 receptors have been described previously.[24] Cells were maintained in an α-minimum essential medium supplemented with penicillin (100 μg/mL), streptomycin (100 μg/mL), and 10% fetal bovine serum. The cells were seeded in 48-well tissue culture plates at a density of 3.0×10^4 cells/well and cultured for 1 day. The cells were washed three times with phosphate-buffered saline containing 0.1% BSA and incubated with 1.0 nM of [^{3}H]BK and test compounds for 2 h at 4 °C in 0.25 mL of binding buffer III (20 mM HEPES, 125 mM *N*-methyl-D-glucamine, 5.0 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.05 mM bacitracin, 5 μM enalaprilat and 0.1% BSA, pH 7.2). All experiments were carried out three times. Nonspecific binding was determined in the presence of 1 μM unlabeled BK. At the end of the incubation, the buffer was aspirated, and the cells were washed twice with ice-cooled phosphate-buffered saline containing 0.1% BSA. The specific binding was calculated by subtracting the nonspecific binding,

determined in the presence of 1 μM unlabeled BK, from the total binding. Bound radioactivity was determined by solubilizing with 1% sodium doedecyl sulfate containing 0.05 N NaOH and quantified in a liquid scintillation counter.

BK−Induced Bronchoconstriction in Guinea Pigs. Male Hartley guinea pigs weighing 470−750 g (from Charles River Japan, Inc.) were fasted overnight and anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg). Then, the trachea and jugular vein were cannulated. The animals were ventilated at a tidal volume of 10 mL/kg with a frequency of 60 breaths/min through the tracheal cannula. To suppress spontaneous respiration, alcuronium chloride (0.5 mg/kg) was administered intravenously through the jugular vein cannula. Then, propranolol (10 mg/kg) was also administered subcutaneously. After 10 min, BK (5 μg/kg, dissolved in saline with 0.1% BSA) was administered intravenously through the jugular vein cannula. Bronchoconstriction was measured by the modified Konzett and Rossler method[36] as the peak increase of pulmonary insufflation pressure (PIP).[37] Each dose of the compound dissolved in 5% (w/v) citric acid solution or vehicle was administered through the same cannula 25 min after the first BK administration. BK was administered again 5 min after the drug injection, and then the bronchoconstriction was measured in the same manner. A 0% response was determined as PIP before the administration of BK, and the 100% response was determined as the first BK-induced bronchoconstriction before drug administration. The percent response was calculated from the following formula: % response = $(\Delta PIP_{after\ drug}/\Delta PIP_{before\ drug}) \times 100$. The efficacy of the drug was expressed as % inhibition which was calculated from the values of % responses of drug-treated and vehicle groups as follows: % inhibition = $(1 - \%\ response_{drug}/\%\ response_{vehicle}) \times 100$.

Agonist-Induced Inositol Phosphates Formation. Inositol phosphate (IPs) formation was measured essentially as described previously.[24] CHO cells expressing the human B_2 receptor were seeded in 12-well plates at density of 1×10^5 cells/well and cultured for 1 day. The cells were labeled with [^{3}H]inositol (1 μCi/mL) for 24 h. The cells were washed twice with PBS containing 0.2% BSA and incubated with the same solution for 30 min and then with PBS containing 0.2% BSA and 10 mM LiCl for 30 min at 37°C. Agonist stimulation was started by replacing the medium with fresh PBS containing 0.2% BSA, 10 mM LiCl, and test compounds. The reaction was terminated by 5% (w/v) trichloroacetic acid after incubation for 30 min at 37 °C. Separation of [^{3}H]inositol phosphates was carried out by Bio-Rad AG 1-X8 chromatography essentially as described elsewhere.[38] A mixture of ^{3}H-labeled inositol monophosphate (IP_1), inositol bisphosphate (IP_2), and inositol trisphosphate (IP_3) was eluted from the column with 0.1 M formic acid/1.0 M ammonium formate. The radioactivity in the eluates was determined by a liquid scintillation spectrometer. The agonist-induced IPs formation was calculated by subtracting the control radioactivity determined in the absence of the compound. The efficacy of the compound was expressed as the relative agonistic activity in IPs formation compared to that of BK (10 nM).

Statistical Analysis. The results are expressed as the mean ± SEM, and statistical significance between groups was analyzed by Student's *t* test. IC_{50} values were obtained by using nonlinear curve-fitting methods with a computer program developed in-house.

Supporting Information Available: Physical data of **6b**, **6d−h**, **11b−h**, **12a**,**b**, **13b−h**, **14**, **25b**,**c**, **26b**,**c**, **27c**, **28**, **29a−c**, **30a−c**, **31a**,**b**, **32**, **33a**,**c**, **34c**, **35a**,**c**, **36**, **37b**,**c**, **38b**,**c**, **39**, **40a−c**, **41a−c**, **42a**,**b**, **43**, **44a**,**c**, **45**, **46b**,**c**, and **48**. This material is available free of charge via the Internet at http://pubs.acs.org.

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