Discovery of the First Non-Peptide Full Agonists for the Human Bradykinin B_2 Receptor Incorporating 4-(2-Picolyloxy)quinoline and 1-(2-Picolyl)benzimidazole Frameworks

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In the course of our studies on non-peptide bradykinin (BK) B_2 receptor ligands, it was suggested that the 4-substituent of the quinoline ring may play a critical role in determining binding affinities for human and guinea pig B_2 receptors, as well as agonist/antagonist properties. We carried out an extensive investigation to elucidate the structure−activity relationships (SAR) for this key pharmacophore. Introduction of lower alkoxy groups to the 4-position of the quinoline ring of **3** led to the identification of 4-ethoxy derivative **22b** as a unique partial agonist. This compound significantly stimulated inositol phosphates (IPs) formation in Chinese hamster ovary cells expressing the cloned human B_2 receptor at concentrations greater than 10 nM and displayed one-tenth of the intrinsic activity of BK. The agonist activity of **22b** was selective for the B_2 receptor and was inhibited by selective peptide and non-peptide B_2 antagonists. On the other hand, **22b** strongly suppressed BK-induced IPs formation through the cloned human B_2 receptor. Further studies on the key pharmacophore led to identification of a 2-picolyloxy moiety as a powerful agonist switch, leading to the discovery of a potent and efficacious non-peptide B_2 agonist, **19a**. Successive optimization of the acyl side chain afforded **38**, which exhibited full agonist activity on stimulation of IPs formation. Furthermore, this strategy could be applied successfully to the benzimidazole series. The representative 1-(2-picolyl)benzimidazole derivative **47c** increased PGE_2 production at a 1 μM concentration to the same level as the maximum effect of BK. Thus, we have established the medicinal chemistry modifications required to convert our highly potent non-peptide B_2 antagonists to agonists with potent efficacy.

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]). Kinins are highly potent agonists of G-protein-coupled cell surface receptors, designated as B_2 receptors, which are expressed constitutively in many tissues and are thought to mediate most of the biological actions of BK.[1,2]

BK exhibits highly potent and diverse biological activities through B_2 receptors, such as bronchoconstriction, vasodilation, plasma extravasation, stimulation of nociceptive neurons, release of various mediators and cytokines, and growth stimulation of small-cell lung cancer.[3−5] On the basis of these strong proinflammatory properties, BK is believed to play important roles in a variety of inflammatory diseases represented by asthma, rhinitis, brain edema, and hyperalgesia.[1,3,6] On the other hand, certain beneficial effects of BK are also mediated by B_2 receptors. It is well accepted that the antihypertensive effect of angiotensin converting enzyme (ACE) inhibitors is partly attributed to increased BK levels.[7] Additionally, blockade of degradation of BK was reported to protect cardiac tissue against ischemic damage.[8] Furthermore, a representative peptide B_2 agonist, RMP-7 (Cereport, [Hyp[3], Thi[5]-4-Me-Tyr[8]Ψ(CH_2NH)]-BK), was shown to enhance the delivery of antitumor agent to the brain.[9,10] Therefore, the development of specific B_2 receptor agonists and antagonists has been of great importance for investigating the pathophysiological roles of BK and for developing novel classes of therapeutic drugs.

We earlier reported the first selective and orally active non-peptide B_2 receptor antagonists with low nanomolar to subnanomolar binding affinities for both the guinea pig and cloned human B_2 receptors (**1**−**4**, Chart 1).[11−15] Subsequent investigations aimed at development of novel therapeutic drugs for iv use led us to identify highly potent antagonists with improved aqueous solubility that significantly inhibited BK-induced bronchoconstriction, even at 1−10 μg/kg by intravenous administration (**5**, **6**, Chart 1, refs 16 and 17) as well as partial agonists, represented by 4-(1-piperidino)quinoline derivative **7**. These studies suggested the critical role of the 4-substituent on the quinoline ring to determine binding affinities for human and guinea pig B_2 receptors and agonist/antagonist properties. In this article, we disclose results from extensive investigations to eluci-

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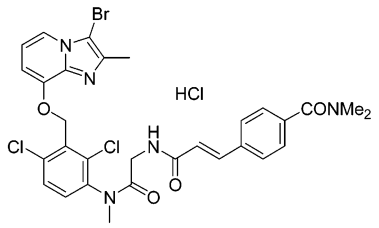
Chart 1. Representative Fujisawa Non-Peptide B_2 Antagonists and Non-Peptide B_2 Partial Agonist



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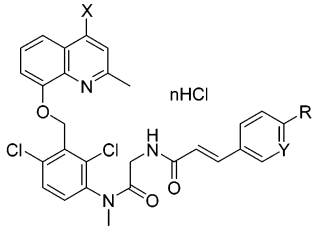
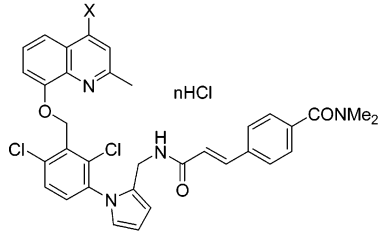
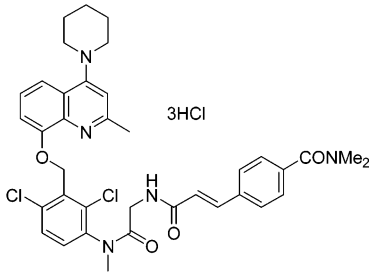


2: X=H, Y=N, R=NHAc, n=0
3: X=H, Y=CH, R=CONHMe, n=1
6: X=NMe_2, Y=CH, R=CONH-4Py, n=3



4: X=H, n=0
5: X=imidazolyl, n=2



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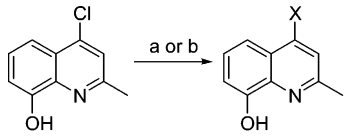
Scheme 1[a]



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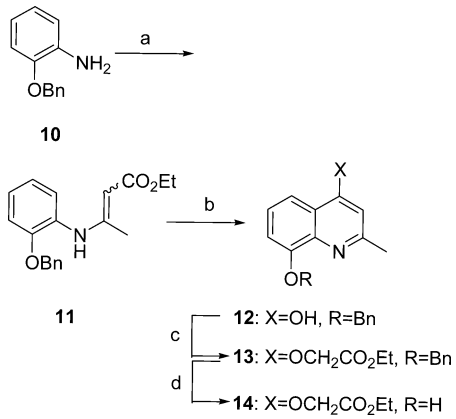
9a: X=OMe **9d**: X=*O*-iso-Pr
9b: X=OEt **9e**: X=*O*-cyclopentyl
9c: X=*O*-n-Pr **9f**: X=OCH_2-2Py

[a] Reagents: (a) 28% NaOMe, MeOH; (b) corresponding alcohols, NaH, 1,3-dimethyl-2-imidazolidinone.

Scheme 2[a]



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11

12: X=OH, R=Bn
13: X=OCH_2CO_2Et, R=Bn
14: X=OCH_2CO_2Et, R=H

[a] Reagents: (a) ethyl acetoacetate, AcOH, benzene; (b) biphenyl, phenyl ether, 235 °C; (c) ethyl bromoacetate, K_2CO_3, DMF; (d) H_2, Pd/C, EtOH, 1,4-dioxane.

date the SAR of this critical pharmacophore, leading to the discovery of a unique partial agonist as well as a powerful agonist switch that enabled us to convert our potent non-peptide B_2 antagonists to efficacious, potent agonists.

Chemistry

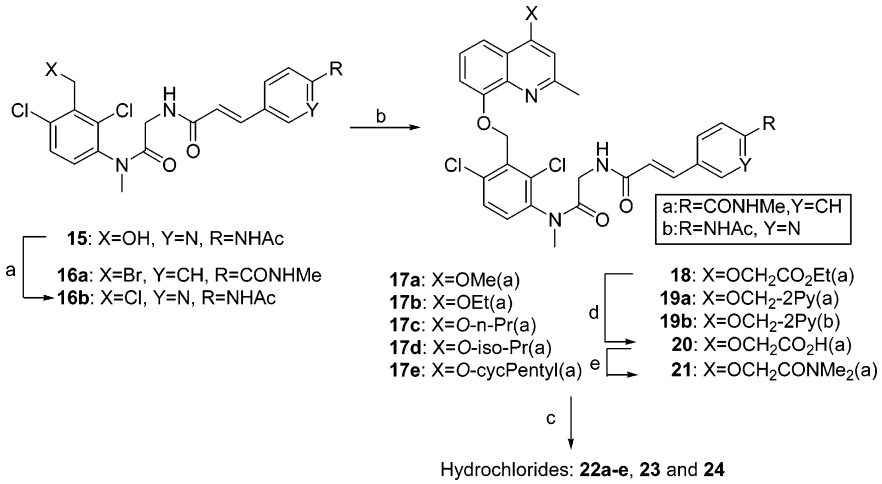
The compounds described in this study are shown in Tables 1−4, and their synthetic methods are outlined in Schemes 1−6.

Preparation of the 4-alkoxy-2-methylquinolinol derivatives **9a**−**f** and **14** are shown in Schemes 1 and 2. Reaction of **8**[16] with the corresponding alcohols yielded the 4-alkoxyquinolinols **9a**−**f** (Scheme 1). The crotonate **11**, which was prepared by condensation of 2-benzyloxyaniline (**10**) with ethyl acetoacetate in benzene in the presence of a catalytic amount of AcOH, was cyclized in Dowtherm at 230 °C to give **12**. The quinoline **12** was condensed with ethyl bromoacetate to give **13**, followed by catalytic deprotection of the benzyl group to yield the 8-hydroxyquinoline **14** (Scheme 2).

Preparation of the 4-alkoxy-2-methylquinoline derivatives **22a**−**e**, **23**, and **24** is shown in Scheme 3. Benzyl alcohol **15**[17] was treated with methanesulfonyl chloride to afford the benzyl chloride **16b**. The quinolinols **9a**−**f** and **14** were coupled with **16a**[13] or **16b** in the presence of K_2CO_3 to give **17a**−**e**, **18**, **19a**, and **19b**. The ethyl ester **18** was hydrolyzed with 1 N NaOH to afford the carboxylic acid **20**, which was condensed with dimethylamine hydrochloride to furnish the amide **21**. The 4-substituted quinoline derivatives **17a**−**e**, **18**, and **21** were treated with 10% HCl in MeOH to afford the corresponding hydrochlorides **22a**−**e**, **23**, and **24**, respectively (Scheme 3).

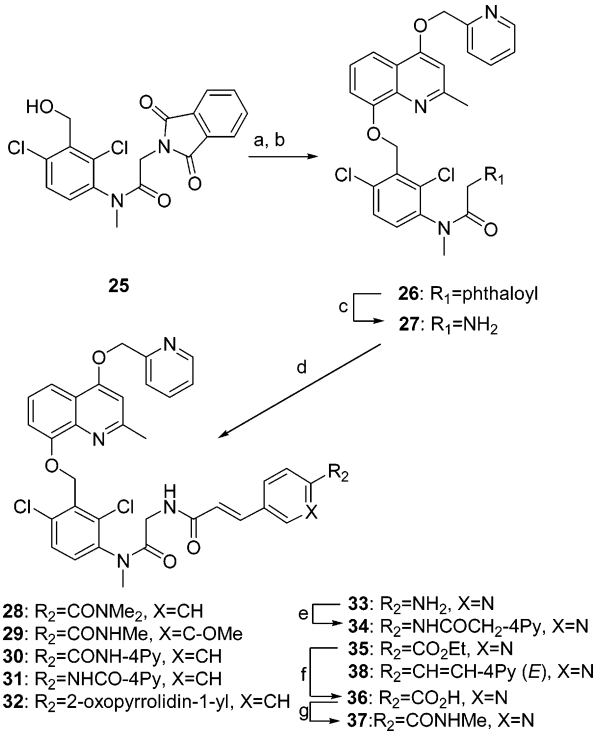
Modifications of the terminal cinnamamide moiety of the 4-(2-pyridinylmethoxy)quinoline derivatives are shown in Scheme 4. The benzyl alcohol **25**[13] was treated with methanesulfonyl chloride followed by coupling with the quinolinol **9f**. Removal of the *N*-phthaloyl group of **26** with hydrazine monohydrate and coupling with (*E*)-4-(substituted)cinnamic acids or (*E*)-3-[6-(substituted)-pyridin-3-yl]acrylic acids afforded the corresponding cinnamamides **28**−**33**, **35**, and **38**. The amine **33** was treated with 4-pyridylacetyl chloride to afford the amide **34**. The acid **36**, which was obtained by saponification of the ester **35**, was condensed with methylamine hydrochloride to furnish the amide **37**.

Scheme 3[a]



[a] Reagents: (a) MsCl, Et_3N, CH_2Cl_2; (b) 4-substituted quinolinols (**9a**−**f** and **14**), K_2CO_3, DMF; (c) 10% HCl−MeOH; (d) 1 N NaOH, EtOH; (e) Me_2NH·HCl, WSCD, HOBt, DMF.

Scheme 4[a]

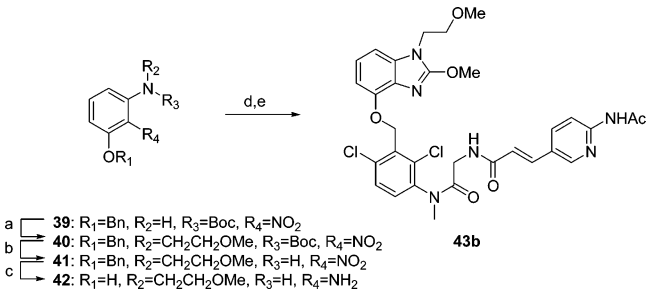


[a] Reagents: (a) MsCl, Et_3N, CH_2Cl_2; (b) **9f**, K_2CO_3, DMF; (c) N_2H_4·H_2O, EtOH; (d) (*E*)-4-(substituted)cinnamic acids or (*E*)-3-[6-(substituted)pyridin-3-yl]acrylic acids, WSCD·HCl, HOBt, DMF; (e) 4-pyridylacetyl chloride, Et_3N, CH_2Cl_2; (f) 1 N NaOH, EtOH; (g) $MeNH_2$·HCl, WSCD, HOBt, DMF.

Alkylation of **39** with 2-chloroethyl methyl ether using K_2CO_3 as a base gave **40**. After removal of the *N*-Boc group of **40**, compound **41** was hydrogenated to give the diamine **42**. Alkylation of the phenol **42** with the benzyl chloride **16b** in the presence of NaH and subsequent cyclization with tetramethyl orthocarbonate gave the 2-methoxybenzimidazole derivative **43b** (Scheme 5).

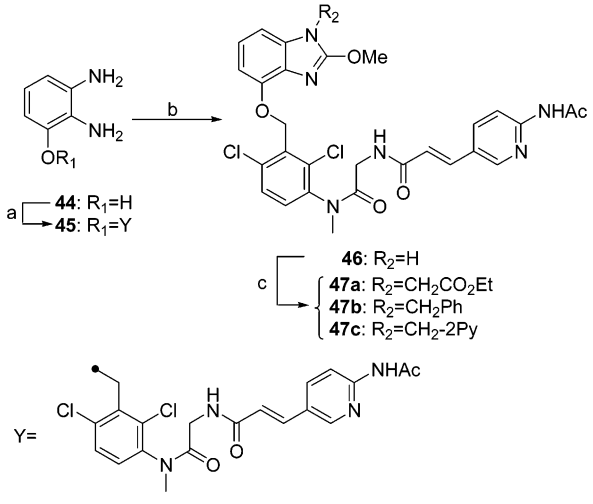
Preparation of the 3-substituted-2-methoxybenzimidazole derivatives **47a**,**b** and **47c** are shown in Scheme 6. Alkylation of 2,3-diaminophenol (**44**) with the benzyl chloride **16b** in the presence of NaH and subsequent cyclization with tetramethyl orthocarbonate gave the 2-methoxybenzimidazole derivative **46**. Alkylation of **46** with the appropriate alkyl bromide or alkyl chloride gave the 3-substituted 2-methoxybenzimidazole derivatives **47a**,**b** or **47c**, respectively.

Scheme 5[a]



[a] Reagents: (a) 2-chloroethyl methyl ether, K_2CO_3, Bu_4NI, DMF; (b) 4 N HCl in EtOAc; (c) H_2, Pd/C, EtOH, 1,4-dioxane; (d) NaH, **16b**, DMF; (e) $C(OMe)_4$, AcOH.

Scheme 6[a]

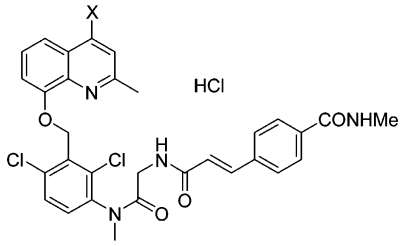


[a] Reagents: (a) NaH, **16b**, DMF; (b) $C(OMe)_4$, AcOH; (c) alkyl halide, K_2CO_3, DMF.

Biology

All compounds were tested for inhibition of the specific binding of [^{3}H]BK to B_2 receptors in guinea pig ileum membrane preparations, as previously reported,[11,12,18−20] and they were also evaluated for inhibi-

Table 1. Binding of 4-Alkoxy Derivatives to Guinea Pig and Cloned Human B_2 Receptors



		IC_{50} (nM)	
compd	X	GP ileum[a]	cloned human B_2[b]
3	H	0.51[c]	1.1[c]
22a	OMe	0.23	8.1
22b	OEt	0.19	34
22c	*O*-*n*-propyl	1.2	77
22d	*O*-isopropyl	1.2	13
22e	*O*-cyclopentyl	25	61

[a] Concentration required to inhibit specific binding of [^{3}H]BK (0.06 nM) to the B_2 receptor 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 Experimental Section for further details. [b] Concentration required to inhibit specific binding of [^{3}H]BK (1.0 nM) to the human B_2 receptor that was expressed in CHO cells by 50%. Values are expressed as the average of at least three determinations, with variation in individual values of <15%. See Experimental Section for further details. [c] Previously published (see ref 13).

tion of the specific binding of [^{3}H]BK to cloned human B_2 receptors expressed in Chinese hamster ovary (CHO) cells.[19] Furthermore, some compounds were examined for their B_2 agonistic activity by measuring both agonist-induced inositol phosphates (IPs) formation in CHO cells expressing human B_2 receptors and agonist-induced PGE_2 production in human fibroblasts, WI-38 cells.

Results and Discussion

Introduction of nitrogen-containing heteroaromatic moieties and aliphatic amines at the 4-position of the quinoline ring enabled us to identify highly potent B_2 antagonists with improved aqueous solubility for iv use as well as the first partial agonists. These results prompted us to carry out a more extensive investigation to elucidate the SAR for this key pharmacophore to determine binding profiles and agonist/antagonist properties.

At first, we introduced several alkoxy groups to the 4-position of the quinoline ring of **3**. As shown in Table 1, these 4-alkoxyquinoline derivatives showed remarkably higher affinities for the guinea pig B_2 receptor than for the cloned human B_2 receptor. It is interesting that this species difference was in marked contrast to that observed with 4-alkylamino- or 4-nitrogen-containing heteroaromatic derivatives.[17] It seemed that small 4-alkoxy groups are favorable for interaction with guinea pig B_2 receptors, while they are unfavorable for human B_2 receptor.

Detailed pharmacological investigation of the in vitro activity of 4-ethoxy derivative **22b** to cloned human B_2 receptors revealed a highly interesting profile. In response to this compound, a significant increase in IPs formation was observed in CHO cells expressing the cloned human B_2 receptor at a concentration of 10 nM (Figure 1). The maximum stimulation was achieved in

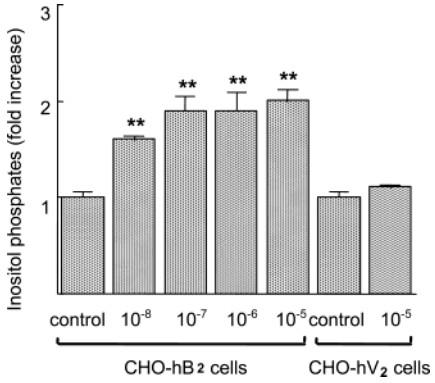


Figure 1. Agonist activity of the compound **22b**.

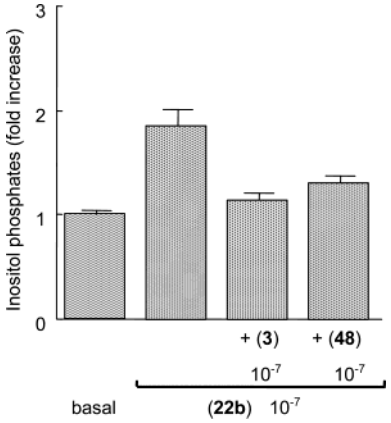
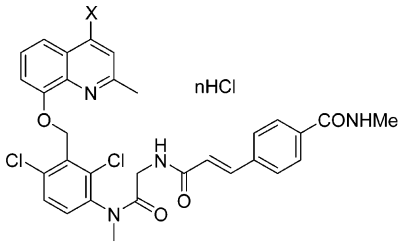


Figure 2. Effect of bradykinin antagonists on compound **22b** induced inositol phosphate formation.

the range from 100 nM to 10 μM, enhancing IPs formation twice over the basal level. The intrinsic activity was about one-tenth of the maximum effect elicited by BK. **22b** showed no stimulatory effect on IPs formation in CHO cells expressing the cloned human vasopressin V_2 receptor instead of the B_2 receptor (Figure 1). In addition, the enhancement of IPs formation by 100 nM **22b** was significantly inhibited by the same concentrations of the representative second-generation peptide B_2 antagonist **48** (HOE140; icatibant; [D-Arg^0,Hyp^3,Thi,^{5}D-Tic^7,Oic^8]BK) or our non-peptide B_2 antagonist **3** (Figure 2). These results clearly indicate that the effect of **22b** on IPs formation is mediated by the human B_2 receptor. On the other hand, **22b** inhibited the IPs formation elicited by 1−100 nM of BK (Figure 3). It is noteworthy that the maximum effect of BK was remarkably decreased by **22b**. Preliminary studies revealed that the agonistic activity of **22b** partly remained even after removal of the ligand by washing the cells, suggesting a tight binding profile of **22b** to the cloned human B_2 receptor (data not shown). This property might contribute to the strong inhibitory activity against a high concentration of BK, although other possibilities, such as desensitization of the receptor, cannot be excluded at this stage.

As the next step, we introduced several functional groups on the alkoxy moiety, aiming at enhancing the efficacy of our non-peptide B_2 partial agonists (Table 2). In addition to a direct measurement of the formation of IPs in CHO cells expressing the human B_2 receptor, we also used a functional assay system to determine the effect on PGE_2 production in human fibroblast WI-38 cells. BK showed the maximum effect on the formation

Table 2. Binding and B_2 Agonistic Activities of 4-Alkoxy Derivatives-1



			IC_{50} (nM)		relative agonistic activity (%) in IPs formation compared to BK (10 nM)[c]			relative agonistic activity (%) in PGE_2 production compared to BK (100 nM)[d]	
compd	X	*n*	GP ileum[a]	cloned human B_2[b]	0.1 μM	1 μM	10 μM	1 μM	10 μM
23	OCH_2COOEt	1	20	2.6	NT[e]	53.7 ± 3.3	66.4 ± 5.0	NT[e]	NT[e]
24	OCH_2CONMe_2	1	30	3.7	NT[e]	30.8 ± 0.9	24.4 ± 2.1	NT[e]	NT[e]
20	OCH_2COOH	0	50	>1000	NT[e]	NT[e]	NT[e]	NT[e]	NT[e]
19a	OCH_2-2Py	0	2.3	0.41	51.3 ± 2.5	59.5 ± 3.5	56.9 ± 2.3	81.2 ± 3.4	140 ± 11.3

[a] Concentration required to inhibit specific binding of [^{3}H]BK (0.06 nM) to the B_2 receptor 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 Experimental Section for further details. [b] Concentration required to inhibit specific binding of [^{3}H]BK (1.0 nM) to the human B_2 receptor that was expressed in CHO cells by 50%. Values are expressed as the average of at least three determinations, with variation in individual values of <15%. See Experimental Section for further details. [c] IPs production was measured essentially as described previously.[19] See Experimental Section for further details. [d] Human fibroblasts (WI-38 cell) were used. After incubation, the supernatant was collected and the PGE_2 level included therein was measured by a PGE_2 EIA kit. See Experimental Section for further details. [e] NT, not tested.

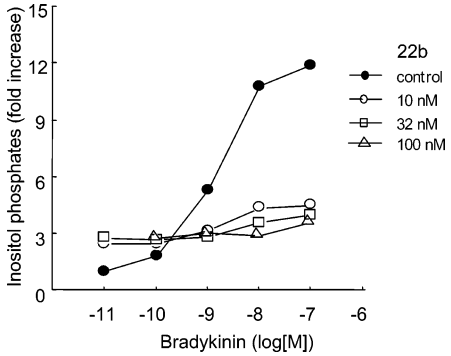


Figure 3. Effect of **22b** on BK-induced inositol phosphate formation in CHO cells expressing the human B_2 receptor.

of IPs at 10 nM and on the production of PGE_2 at 100 nM. Ester derivative **23** stimulated IPs formation more efficaciously than **22b**. It exhibited about a half and two-thirds efficacy of the maximum effect of BK at concentrations of 1 and 10 μM. The dimethylamide derivative **24** also showed significant agonistic activities in the same concentration range, albeit with lower efficacies, and the carboxylic acid **20** completely lost binding affinity for the human B_2 receptor. In contrast, introduction of a 2-pyridyl moiety led to the potent agonist **19a**, which afforded half the efficacy of BK for IPs formation at 0.1 μM, as well as full agonist activity for PGE_2 production at 10 μM. Since we disclosed **19a** as the first non-peptide B_2 agonist in 1997,[21] this compound has been used worldwide as a probe and has been shown to selectively mimic BK activity mediated by B_2 receptors both in vitro and in vivo. Extensive reports on the pharmacology of **19a** have been published by others and us.[21−31] According to these reports, the binding mode of **19a** to the B_2 receptor seems to depend on the species, tissues, and experimental conditions.[25]

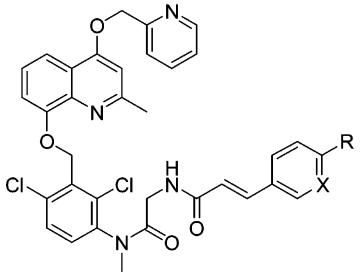
Table 3 summarizes the results of optimization studies on the acyl side chain of **19a**. These results indicated that a variety of side chains could be accommodated in the agonist binding pocket and that they could also modify the efficacy. The most efficacious compound, **38**, was the first example to afford full agonist activity for IPs formation. Thus, we identified the 4-(2-picolyloxy) moiety on the quinoline ring as a powerful agonist switch.

Furthermore, we examined the applicability of this strategy to other frameworks. The 2-methoxybenzimidazole series was selected as a model of our non-peptide B_2 ligands, incorporating five to six condensed ring systems. The representative agonist pharmacophores and related substituents were introduced at the 1-position of the benzimidazole ring. As shown in Table 4, an ester derivative **47a** exhibited about 90% efficacy at 1 μM concentration and full agonist activity at 10 μM on PGE_2 production, while a methoxyethyl derivative **43b** also significantly increased PGE_2 production from 1 μM with lower efficacy. The 1-benzyl derivative **47b** showed its maximum efficacy, 80% of BK, at 1 μM. Finally, the 1-(2-picolyl) derivative **47c** afforded full agonist activity on PGE_2 production at 1 μM concentration. These results indicated that this strategy has a wide range of applicability. Thus, we have established the medicinal chemistry to convert our highly potent non-peptide B_2 antagonists to agonists with strong efficacies.

Conclusion

We carried out extensive investigations to elucidate the structural features of the key pharmacophore to determine the species difference in terms of binding affinities and agonist/antagonist properties. Introduction of lower alkoxy groups to the 4-position of the quinoline ring of **3** resulted in increasing affinities for the guinea pig B_2 receptor and decreasing affinities for human B_2 receptors, leading to partial agonists, as represented by the 4-ethoxy derivative **22b**. This compound significantly stimulated IPs formation in CHO cells expressing cloned human B_2 receptors, from a concentration of 10 nM with about 10% of the intrinsic activity of BK. Its agonistic activity was selective for the human B_2 receptor and was inhibited by the

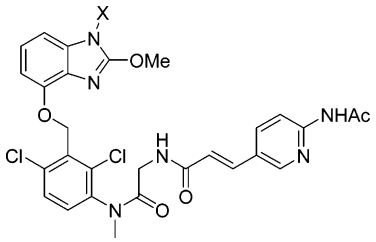
Table 3. Binding and B_2 Agonistic Activities of 4-(2-Picolyloxy)quinolines



compd	X	R	IC_{50} (nM) GP ileum[a]	IC_{50} (nM) cloned human B_2[b]	relative agonistic activity (%) in IPs formation compared to BK (10 nM)[c] 0.1 μM	1 μM
19a	CH	CONHMe	2.3	0.41	51.3 ± 2.5	59.5 ± 3.5
28	CH	$CONMe_2$	NT[d]	1.4	22.3 ± 1.2	34.3 ± 1.7
29	C−OMe	CONHMe	NT[d]	0.69	54.9 ± 6.4	73.9 ± 4.4
30	CH	CONH-4Py	NT[d]	0.40	36.7 ± 4.1	47.4 ± 7.1
31	CH	NHCO-4Py	6.1	0.56	42.3 ± 2.4	26.9 ± 2.6
32	CH	2-oxopyrrolidin-1-yl	NT[d]	0.83	42.9 ± 9.0	67.4 ± 5.4
19b	N	NHAc	1.2	1.2	43.4 ± 10.1	57.0 ± 11.8
34	N	$NHCOCH_2$-4Py	NT[d]	2.4	39.8 ± 2.7	40.3 ± 5.9
37	N	CONHMe	NT[d]	1.4	50.5 ± 8.4	57.6 ± 8.5
38	N	HC═CH-4Py (*E*)	NT[d]	0.36	81.2 ± 3.1	99.3 ± 1.9

[a] Concentration required to inhibit specific binding of [^{3}H]BK (0.06 nM) to the B_2 receptor 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 Experimental Section for further details. [b] Concentration required to inhibit specific binding of [^{3}H]BK (1.0 nM) to the human B_2 receptor that was expressed in CHO cells by 50%. Values are expressed as the average of at least three determinations, with variation in individual values of <15%. See Experimental Section for further details. [c] IPs production was measured essentially as described previously.[19] See Experimental Section for further details. [d] Human fibroblasts (WI-38 cell) were used. After incubation, the supernatant was collected and the PGE_2 level included therein was measured by a PGE_2 EIA kit. See Experimental Section for further details.

Table 4. Binding and B_2 Agonistic Activities of 1-Substituted Benzimidazoles



compd	X	IC_{50} (nM) GP ileum[a]	IC_{50} (nM) cloned human B_2[b]	relative agonistic activity (%) in PGE_2 production compared to BK (100 nM)[c] 1 μM	10 μM
43a	Me	0.65[d]	4.2[d]	4.2 ± 3.5	24.2 ± 4.3
43b	CH_2CH_2OMe	4.6	3.5	38.3 ± 13.0	54.8 ± 11.0
47a	CH_2COOEt	4.2	0.91	90.0 ± 3.5	121 ± 18.5
47b	CH_2Ph	26	2.5	84.5 ± 2.3	73.9 ± 8.5
47c	CH_2−2Py	6.3	0.68	114 ± 2.4	89.5 ± 15.8

[a] Concentration required to inhibit specific binding of [^{3}H]BK (0.06 nM) to the B_2 receptor 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 Experimental Section for further details. [b] Concentration required to inhibit specific binding of [^{3}H]BK (1.0 nM) to the human B_2 receptor that was expressed in CHO cells by 50%. Values are expressed as the average of at least three determinations, with variation in individual values of <15%. See Experimental Section for further details. [c] IPs production was measured essentially as described previously.[19] See Experimental Section for further details. [d] Previously published (see ref 13).

selective B_2 antagonists **48** and **3**. On the other hand, **22b** inhibited IPs formation elicited by BK through the human B_2 receptor, remarkably suppressing the efficacy of a high concentration of BK. Further SAR studies on the key pharmacophore allowed us to identify a 2-picolyloxy moiety as a powerful agonist switch, leading to the discovery of the potent and efficacious non-peptide B_2 agonist **19a**. Successive optimization of the acyl side chain afforded **38**, which exhibited full agonist activity on the second messenger, IPs formation. Furthermore, this strategy could also be applied successfully to the benzimidazole series. The representative 1-(2-picolyl)-benzimidazole derivative **47c** increased PGE_2 production at 1 μM to the same level as the maximum effect of BK. Thus, we have established the medicinal chemistry to convert our highly potent non-peptide B_2 antagonists to agonists with strong efficacies.

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 the

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 thin-layer chromatography was performed on precoated plates, Kieselgel 60F_{254} (E. Merck, AG, Darmstadt, Germany). Silica gel flash chromatography was performed with Kieselgel 60 (230−400 mesh) (E. Merck, AG, Darmstadt, Germany). Yields were not optimized.

4-Methoxy-2-methyl-8-quinolinol (9a). To a solution of 4-chloro-2-methyl-8-quinolinol (**8**)[16] (49.0 g, 253 mmol) in 1,3-dimethyl-2-imidazolidinone (DMI, 490 mL) was added a solution of 28% NaOMe in MeOH (244 mL, 1.265 mol) at ambient temperature, and the mixture was refluxed for 5 h. The cooled mixture was adjusted to pH 7 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 resulting residue was crystallized from hexane to afford **9a** (39.0 g, 81.6%) as colorless crystals: mp 111−112 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.68 (s, 3H), 4.01 (s, 3H), 6.62 (s, 1H), 7.10 (d, J = 8 Hz, 1H), 7.30 (dd, J = 8, 8 Hz, 1H), 7.58 (d, J = 8 Hz, 1H). Anal. ($C_{11}H_{11}NO_2$) C, H, N.

4-Ethoxy-2-methyl-8-quinolinol (9b). To EtOH (2 mL) was added 60% NaH in oil (200 mg, 5.0 mmol) portionwise in an ice/water bath under nitrogen, and the mixture was stirred at the same temperature for 15 min. 4-Chloro-2-methyl-8-quinolinol (**8**) (194 mg, 1.00 mmol) was added, and the solvent was evaporated in vacuo. DMI (2 mL) was added to the residue, and then the mixture was stirred at 100 °C for 23 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 resulting residue was crystallized from IPE to afford **9b** (142 mg, 69.9%) as colorless crystals: mp 85−86 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.55 (t, J = 7.5 Hz, 3H), 2.66 (s, 3H), 4.23 (q, J = 7.5 Hz, 2H), 6.61 (s, 1H), 7.10 (d, J = 8 Hz, 1H), 7.30 (dd, J = 8, 8 Hz, 1H), 7.59 (d, J = 8 Hz, 1H). Anal. ($C_{12}H_{13}NO_2$) C, H, N.

Compounds **9c**−**f** were prepared following the procedure described above for **9b**.

Ethyl (2*E*)-3-[2-(Benzyloxy)anilino]-2-butenoate or Ethyl (2*Z*)-3-[2-(Benzyloxy)anilino]-2-butenoate (11). A solution of 2-benzyloxyaniline (**10**) (15.0 g, 75.3 mmol), ethyl acetoacetate (10.3 g, 10.1 mL, 79.2 mmol), and AcOH (1.5 mL) in benzene (45 mL) was refluxed for 6 h, removing water with a Dean−Stark apparatus. The mixture was then cooled to room temperature and concentrated in vacuo. The residue was purified by flash silica gel chromatography (hexane/EtOAc, 5:1) to afford **11** (20.9 g, 89.3%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, J = 7 Hz, 3H), 1.99 (s, 3H), 4.16 (q, J = 7 Hz, 2H), 4.73 (s, 1H), 5.11 (s, 2H), 6.88−6.99 (m, 2H), 7.03−7.15 (m, 2H), 7.26−7.40 (m, 3H), 7.47 (d, J = 8 Hz, 2H). Anal. ($C_{19}H_{21}NO_3$) C, H, N.

8-(Benzyloxy)-2-methyl-4-quinolinol (12). To a mixture of biphenyl (9.6 g) and phenyl ether (22.4 mL) was added dropwise crotonate **11** (20 g, 64.2 mmol) at 230−235 °C over 15 min. After 1 h of being stirred at 235 °C, the reaction mixture was cooled at room temperature. The mixture was diluted with hexane (200 mL), and the precipitate was collected by vacuum filtration. The residue was purified by flash silica gel chromatography ($CHCl_3$/MeOH, 10:1) followed by crystallization from CH_3CN to afford **12** (4.21 g, 24.8%) as pale-brown crystals: mp 155−164 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (s, 3H), 5.38 (s, 2H), 5.90 (s, 1H), 7.13 (dd, J = 8, 8 Hz, 1H), 7.22 (d, J = 8 Hz, 1H), 7.28−7.43 (m, 3H), 7.53 (d, J = 8 Hz, 2H), 7.57 (d, J = 8 Hz, 1H). Anal. ($C_{17}H_{15}NO_2$) C, H, N.

Ethyl {[8-(Benzyloxy)-2-methyl-4-quinolinyl]oxy}acetate (13). To a mixture of 8-(benzyloxy)-2-methyl-4-quinolinol (**12**) (1.50 g, 5.65 mmol) and K_2CO_3 (860 mg, 6.22 mmol) in DMF (15 mL) was added ethyl bromoacetate (0.69 mL, 6.22 mmol) in an ice/water bath under nitrogen. After 10 min, the reaction mixture was stirred at ambient temperature for 4 h. The mixture was then partitioned between CH_2Cl_2 and water. The organic layer was washed with water, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was washed with Et_2O to afford **13** (1.47 g, 73.9%) as a pale-yellow solid: mp 138−140 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (t, J = 7.5 Hz, 3H), 2.74 (s, 3H), 4.31 (q, J = 7.5 Hz, 2H), 4.81 (s, 2H), 5.43 (s, 2H), 6.53 (s, 1H), 7.02 (d, J = 8 Hz, 1H), 7.22−7.40 (m, 4H), 7.51 (d, J = 8 Hz, 2H), 7.79 (d, J = 8 Hz, 1H). Anal. ($C_{21}H_{21}NO_4$) C, H, N.

Ethyl [(8-Hydroxy-2-methyl-4-quinolinyl)oxy]acetate (14). A mixture of **13** (1.30 g, 3.70 mmol) and 10% palladium on carbon (130 mg) in a mixture of EtOH (8 mL) and 1,4-dioxane (7 mL) was hydrogenated at ambient temperature. After completion, the catalyst was removed by filtration, and solvent was evaporated in vacuo. The resulting residue was washed with Et_2O to afford **14** (539 mg, 55.8%) as a pale-yellow solid: mp 97−98 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.23 (t, J = 7.5 Hz, 3H), 2.60 (s, 3H), 4.22 (q, J = 7.5 Hz, 2H), 5.07 (s, 2H), 6.92 (s, 1H), 7.04 (d, J = 8 Hz, 1H), 7.34 (dd, J = 8, 8 Hz, 1H), 7.52 (d, J = 8 Hz, 2H). Anal. ($C_{14}H_{15}NO_4$) C, H, N.

Compound **42** was prepared following the procedure described above for **14**.

(2*E*)-3-[6-(Acetylamino)-3-pyridinyl]-*N*-{2-[3-(chloromethyl)-2,4-dichloromethylanilino]-2-oxoethyl}-2-propenamide (16b). To a solution of **15** (5.36 g, 11.9 mmol) and Et_3N (2.40 g, 23.8 mmol) in dry DMF (30 mL) was added methanesulfonyl chloride (1.90 g, 16.6 mmol) in an ice/water bath under nitrogen. After 30 min, the reaction mixture was stirred at ambient temperature for 18 h. The mixture was partitioned between $CHCl_3$ and water, and the organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo. The residue was washed with EtOAc to afford **16b** (5.08 g, 91.1%) as a pale-yellow amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.10 (s, 3H), 3.10 (d, J = 5 Hz, 3H), 3.45 (dd, J = 17, 3 Hz, 1H), 3.74 (dd, J = 17, 3 Hz, 1H), 4.96 (s, 2H), 6.76 (d, J = 15 Hz, 1H), 7.35 (d, J = 15 Hz, 1H), 7.70−7.40 (m, 2H), 7.98 (d, J = 8 Hz, 1H), 8.11 (d, J = 8 Hz, 1H), 8.23 (t, J = 5 Hz, 1H), 8.45 (d, J = 3 Hz, 1H); MS (ESI) m/z 469, 471 (M + 1). Anal. ($C_{20}H_{19}Cl_3N_4O_3$) C, H, N.

4-((1*E*)-3-{[2-(2,4-Dichloro-3-{[(4-methoxy-2-methyl-8-quinolinyl)oxy]methyl}methylanilino)-2-oxoethyl]amino}-3-oxo-1-propenyl)-*N*-methylbenzamide (17a). To a mixture of **16a** (80.0 mg, 0.156 mmol) and **9a** (31.0 mg, 0.164 mmol) in dry DMF (1.0 mL) was added K_2CO_3 (64.6 mg, 0.467 mmol) at ambient temperature, and the mixture was stirred at the 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 **17a** (76.0 mg, 78.4%) as a colorless amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.67 (s, 3H), 3.00 (d, J = 5 Hz, 3H), 3.26 (s, 3H), 3.15 (dd, J = 17, 4 Hz, 1H), 3.92 (dd, J = 17, 5 Hz, 1H), 4.02 (s, 3H), 5.59 (d, J = 10 Hz, 1H), 5.63 (d, J = 10 Hz, 1H), 6.38 (br d, J = 5 Hz, 1H), 6.52 (d, J = 15 Hz, 1H), 6.65 (s, 1H), 6.76 (br s, 1H), 7.21−7.31 (m, 2H), 7.38 (dd, J = 8, 8 Hz, 1H), 7.43−7.61 (m, 4H), 7.75 (d, J = 8 Hz, 2H), 7.83 (d, J = 8 Hz, 1H); MS (FAB) m/z 621.3 (M + 1). Anal. ($C_{32}H_{30}Cl_2N_4O_5$) C, H, N.

Compounds **17b**−**e**, **18**, and **19a**,**b** were prepared following the procedure described above for **17a**.

[(8-{[2,6-Dichloro-3-(methyl{[((2*E*)-3-{4-[(methylamino)carbonyl]phenyl}-2-propenoyl)amino]acetyl}amino)benzyl]oxy}-2-methyl-4-quinolinyl)oxy]acetic Acid (20). To a solution of **18** (207 mg, 0.298 mmol) in EtOH (2 mL) was added 1 N NaOH (0.36 mL) at ambient temperature, and the mixture was stirred at the same temperature for 2 h. The reaction mixture was adjusted to pH 4 with 1 N HCl and diluted with water. The precipitate was collected by vacuum filtration and washed with water to afford **20** (176 mg, 88.9%) as a colorless solid: mp 233−257 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.54 (s, 3H), 2.78 (d, J = 5 Hz, 3H), 3.17 (s, 3H), 3.51 (dd, J = 17, 5 Hz, 1H), 3.82 (dd, J = 17, 5 Hz, 1H), 4.96 (s, 2H), 5.47 (d, J = 10 Hz, 1H), 5.53 (d, J = 10 Hz, 1H), 6.89

(d, J = 15 Hz, 1H), 6.93 (s, 1H), 7.33−7.50 (m, 3H), 7.60−7.70 (m, 2H), 7.73−7.81 (m, 3H), 7.85 (d, J = 8 Hz, 2H), 8.32 (t, J = 4.5 Hz, 1H), 8.49 (q, J = 4.5 Hz, 1H). Anal. ($C_{33}H_{30}Cl_2N_4O_7$) C, H, N.

Compound **36** was prepared following the procedure described above for **20**.

4-{(1*E*)-3-[(2-{2,4-Dichloro-3-[({4-[2-(dimethylamino)-2-oxoethoxy]-2-methyl-8-quinolinyl}oxy)methyl]methylanilino}-2-oxoethyl)amino]-3-oxo-1-propenyl}-*N*-methylbenzamide (21). To a solution of **20** (82.3 mg, 0.124 mmol), dimethylamine hydrochloride (12.1 mg, 0.148 mmol), and 1-hydroxybenzotriazole (HOBt, 26.7 mg, 0.198 mmol) in dry DMF (1 mL) was added 1-ethoxy-3-[3-(dimethylamino)propyl]-carbodiimide (WSCD, 26.9 mg, 0.173 mmol) in an ice/water bath under nitrogen, and the mixture was stirred at ambient temperature for 14 h. The reaction mixture was partitioned between $CHCl_3$ and water. The organic layer was separated, washed with saturated aqueous $NaHCO_3$, 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 **21** (80.5 mg, 94.0%) as a colorless amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.53 (s, 3H), 2.76 (d, J = 5 Hz, 3H), 2.86 (s, 3H), 3.04 (s, 3H), 3.15 (s, 3H), 3.50 (dd, J = 17, 5 Hz, 1H), 3.80 (dd, J = 17, 5 Hz, 1H), 5.10 (s, 2H), 5.45 (d, J = 9 Hz, 1H), 5.51 (d, J = 9 Hz, 1H), 6.87 (d, J = 15 Hz, 1H), 6.88 (s, 1H), 7.32−7.48 (m, 3H), 7.61−7.69 (m, 2H), 7.73−7.81 (m, 3H), 7.87 (d, J = 8 Hz, 2H), 8.33 (t, J = 5.5 Hz, 1H), 8.48 (q, J = 5.5 Hz, 1H). Anal. ($C_{35}H_{35}Cl_2N_5O_6$) C, H, N.

Compound **37** was prepared following the procedure described above for **21**.

4-((1*E*)-3-{[2-(2,4-Dichloro-3-{[(4-methoxy-2-methyl-8-quinolinyl)oxy]methyl}methylanilino)-2-oxoethyl]amino}-3-oxo-1-propenyl)-*N*-methylbenzamide Hydrochloride (22a). To a solution of **17a** (70.0 mg, 0.113 mmol) in MeOH (2 mL) was added 10% HCl in MeOH (2 mL) at ambient temperature. The reaction mixture was stirred at the same temperature for 10 min. The solution was evaporated in vacuo, and the residue was washed with EtOAc to afford **22a** (73.0 mg, 98.5%) as a pale-yellow amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD) δ 2.99 (s, 3H), 3.00 (br s, 3H), 3.29 (s, 3H), 3.89 (d, J = 17 Hz, 1H), 4.10 (d, J = 17 Hz, 1H), 4.36 (s, 3H), 5.51 (d, J = 10 Hz, 1H), 5.68 (d, J = 10 Hz, 1H), 6.63 (d, J = 15 Hz, 1H), 7.35−7.43 (m, 2H), 7.48−7.59 (m, 6H), 7.70−7.81 (m, 4H), 7.95 (d, J = 8 Hz, 1H). Anal. ($C_{32}H_{30}Cl_2N_4O_5$·HCl) C, H, N.

Compounds **22b**−**e**, **23**, and **24** were prepared following the procedure described above for **22a**.

***N*-[2,4-Dichloro-3-({[2-methyl-4-(2-pyridinylmethoxy)-8-quinolinyl]oxy}methyl)phenyl]-2-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)-*N*-methylacetamide (26). Step 1.** To a solution of **25** (900 mg, 2.29 mmol) and Et_3N (279 mg, 2.76 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise methanesulfonyl chloride (288 mg, 2.51 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 (1.08 g, ∼100%) as a pale-yellow oil.

Step 2. To a suspension of 60% NaH in oil (99.0 mg, 2.48 mmol) in DMF (0.5 mL) was added a solution of **9f** (600 mg, 2.25 mmol) in DMF (5 mL) in an ice/water bath under nitrogen, and the mixture was stirred under the same conditions for 30 min. A solution of methanesulfonate (1.08 g, 2.29 mmol) in dry DMF (25 mL) was added dropwise to the mixture under the same conditions, 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 purified by flash silica gel chromatography ($CHCl_3$/MeOH, 20:1) followed by trituration with EtOAc to afford **26** (772 mg, 53.4%) as a colorless solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.67 (s, 3H), 3.21 (s, 3H), 4.01 (s, 2H), 5.38 (s, 3H), 5.67 (d, J = 10 Hz, 1H), 5.73 (d, J = 10 Hz, 1H), 6.70 (s, 1H), 7.21−7.32 (m, 2H), 7.37 (t, J = 8 Hz, 1H), 7.45 (d, J = 8 Hz, 1H), 7.52 (d, J = 8 Hz, 1H), 7.57 (d, J = 8 Hz, 1H), 7.64−7.80 (m, 3H), 7.80−7.89 (m, 2H), 7.95 (d, J = 8 Hz, 1H), 8.64 (d, J = 6 Hz, 1H); MS (ESI) m/z 641 (M + 1). Anal. ($C_{34}H_{26}Cl_2N_4O_5$) C, H, N.

2-Amino-*N*-[2,4-dichloro-3-({[2-methyl-4-(2-pyridinylmethoxy)-8-quinolinyl]oxy}methyl)phenyl]-*N*-methylacetamide (27). A mixture of **26** (750 mg, 1.20 mmol) and hydrazine monohydrate (117 mg, 2.34 mmol) in EtOH (7.5 mL) was heated under reflux for 4 h. The precipitate was removed by vacuum filtration, and the filtrate was evaporated in vacuo. The residue was dissolved in a mixture of $CHCl_3$ and MeOH (10:1), the precipitate was removed by vacuum filtration, and the filtrate was concentrated in vacuo again. The residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH, 10:1) to afford **27** (390 mg, 65.2%) as a pale-yellow amorphous solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.55 (s, 3H), 2.76 (d, J = 18 Hz, 1H), 3.01 (d, J = 18 Hz, 1H), 3.11 (s, 3H), 3.40−3.60 (m, 1H), 4.10 (br peak, 1H), 5.37−5.63 (m, 4H), 7.07 (s, 1H), 7.33−7.53 (m, 3H), 7.62−7.94 (m, 5H), 8.62 (d, J = 6 Hz, 1H); MS (ESI) m/z 511 (M + 1). Anal. ($C_{26}H_{24}Cl_2N_4O_3$) C, H, N.

4-[(1*E*)-3-[[2-[2,4-Dichloro(methyl)-3-({[2-methyl-4-(2-pyridinylmethoxy)-8-quinolinyl]oxy}methyl)anilino]-2-oxoethyl]amino]-3-oxo-1-propenyl]-*N*,*N*-dimethylbenzamide (28). To a solution of **27** (23.0 mg, 0.045 mmol) in DMF (2 mL) were added (*E*)-4-(*N*,*N*-dimethylcarbamoyl)cinnamic acid (10.8 mg, 0.049 mmol), WSCD·HCl (10.3 mg, 0.054 mmol), and HOBt (9.12 mg, 0.067 mmol) at ambient temperature. After 3 h, this mixture was partitioned between EtOAc and water. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, water (3×), 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 **28** (17.0 mg, 53.0%) as a colorless amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.64 (s, 3H), 2.98 (br s, 3H), 3.10 (br s, 3H), 3.25 (s, 3H), 3.61 (dd, J = 18, 4 Hz, 1H), 3.91 (dd, J = 18, 4 Hz, 1H), 5.40 (s, 2H), 5.64 (s, 2H), 6.51 (d, J = 16 Hz, 1H), 6.71 (s, 1H), 6.75 (t, J = 5 Hz, 1H), 7.21−7.35 (m, 3H), 7.35−7.45 (m, 3H), 7.45−7.64 (m, 5H), 7.76 (dd, J = 8, 8 Hz, 1H), 7.96 (d, J = 8 Hz, 1H), 8.65 (d, J = 6 Hz, 1H); MS (ESI) m/z 712 (M + 1). Anal. ($C_{38}H_{35}Cl_2N_5O_5$) C, H, N.

Compounds **29**−**33**, **35,** and **38** were prepared following the procedure described above for **28**.

(2*E*)-*N*-{2-[2,4-Dichloro(methyl)-3-({[2-methyl-4-(2-pyridinylmethoxy)-8-quinolinyl]oxy}methyl)anilino]-2-oxoethyl}-3-{6-[(4-pyridinylacetyl)amino]-3-pyridinyl}-2-propenamide (34). To an ice-cooled mixture of **33** (90 mg, 0.14 mmol) and Et_3N (856 mg, 0.55 mmol) in CH_2Cl_2 (2 mL) were added 4-pyridylacetyl chloride (53 mg, 0.27 mmol) and nitrogen, and the mixture was stirred at the same temperature for 1 h and allowed to stand at ambient temperature for 1 day. The reaction mixture was poured into water and extracted with $CHCl_3$. 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 give **34** (10 mg, 9%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.65 (s, 3H), 3.25 (s, 3H), 3.63 (dd, J = 18, 4 Hz, 1H), 3.74 (s, 2H), 3.93 (dd, J = 18, 4 Hz, 1H), 5.41 (s, 2H), 5.63 (s, 2H), 6.46 (d, J = 16 Hz, 1H), 6.71 (s, 1H), 6.75 (br peak, 1H), 7.24−7.33 (m, 5H), 7.34 (dd, J = 8, 8 Hz, 1H), 7.40−7.55 (m, 2H), 7.59 (d, J = 8 Hz, 1H), 7.75 (dd, J = 8, 8 Hz, 1H), 7.81 (d, J = 8 Hz, 1H), 7.95 (d, J = 8 Hz, 1H), 8.12−8.21 (m, 2H), 8.31 (d, J = 2 Hz, 1H), 8.59−8.67 (m, 3H); MS (ESI) m/z 776 (M + 1). Anal. ($C_{41}H_{35}Cl_2N_7O_5$) C, H, N.

***tert*-Butyl 3-(Benzyloxy)-2-nitrophenyl(2-methoxyethyl)carbamate (40).** To a solution of *tert*-butyl 3-(benzyloxy)-2-nitrophenylcarbamate (**39**) (600 mg, 1.74 mmol) and 2-chloroethyl methyl ether (666 mg, 6.97 mmol) in dry DMF (9 mL) were added K_2CO_3 (1.20 g, 8.68 mmol) and tetrabutylammonium iodide (60 mg) at ambient temperature under nitrogen, and the reaction mixture was stirred at 100 °C for 6 h. The 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 purified by flash silica gel chromatography (hexane/EtOAc, 3:1) to afford **40** (545 mg, 77.7%) as a pale-yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (br peak, 9H), 3.33 (s, 3H), 3.39−4.03 (m, 4H), 5.19 (br s, 2H), 6.91−7.05 (m, 2H), 7.27−7.45 (m, 6H); MS (ESI) *m*/*z* 403.1. Anal. ($C_{21}H_{26}N_2O_6$) C, H, N.

3-Benzyloxy-*N*-(2-methoxyethyl)-2-nitroaniline (41). A mixture of **40** (542 mg, 1.35 mmol) in 4 N HCl in EtOAc (3 mL, 12 mmol) was stirred at ambient temperature for 1 h. The reaction mixture was evaporated in vacuo. To the residue was added saturated aqueous $NaHCO_3$, and then the mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over $MgSO_4$, and evaporated in vacuo to give **41** (396 mg, 97.3%) as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.35 (q, J = 6 Hz, 2H), 3.40 (s, 3H), 3.61 (t, J = 6 Hz, 2H), 5.16 (s, 2H), 6.21 (t, J = 5 Hz, 1H), 6.30−6.42 (m, 2H), 7.20 (dd, J = 8, 8 Hz, 1H), 7.27−7.47 (m, 5H); MS (ESI) *m*/*z* 303.2 (M + 1). Anal. ($C_{16}H_{18}N_2O_4$) C, H, N.

(2*E*)-3-[6-(Acetylamino)-3-pyridinyl]-*N*-{2-[2,4-dichloro-3-({[2-methoxy-1-(2-methoxyethyl)-1*H*-benzimidazol-4-yl]oxy}methyl)methylanilino]-2-oxoethyl}-2-propenamide (43b). Step 1. To an ice-cooled solution of **42** (232 mg, 1.27 mmol) in DMF (3 mL) was added 60% sodium hydride in oil (53 mg, 1.33 mmol) under nitrogen. After 30 min, **16b** was added, followed by stirring at ambient temperature for 1 h. The reaction mixture was then 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 to give intermediate diamine.

Step 2. To a solution of diamine in AcOH (2 mL) was added tetramethyl orthocarbonate (304 mg, 2.23 mmol) at ambient temperature, and the mixture was stirred at the same temperature for 20 h. The reaction mixture was evaporated in vacuo, and AcOH was azeotropically removed with toluene. The residue was purified by flash silica gel column chromatography ($CHCl_3$/MeOH, 50:1) and by preparative thin-layer chromatography (EtOAc/MeOH, 12:1) to afford **43b** (96.0 mg, 11.5%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.23 (s, 3H), 3.27 (s, 3H), 3.32 (s, 3H), 3.62−3.73 (m, 2H), 3.95 (dd, J = 18, 4 Hz, 1H), 4.13 (t, J = 7 Hz, 2H), 4.20 (s, 3H), 5.65 (s, 2H), 6.46 (d, J = 16 Hz, 1H), 6.67 (t, J = 5 Hz, 1H), 6.83 (t, J = 8 Hz, 1H), 6.92 (d, J = 8 Hz, 1H), 7.10 (dd, J = 8, 8 Hz, 1H), 7.30 (d, J = 8 Hz, 1H), 7.45−7.58 (m, 2H), 7.85 (dd, J = 8, 2 Hz, 1H), 8.21 (d, J = 8 Hz, 1H), 8.35 (d, J = 2 Hz, 1H); MS (ESI) *m*/*z* 655 (M + 1). Anal. ($C_{31}H_{32}Cl_2N_6O_6$) C, H, N.

(2*E*)-3-[6-(Acetylamino)-3-pyridinyl]-*N*-(2-{2,4-dichloro-3-[(2,3-diaminophenoxy)methyl]methylanilino}-2-oxoethyl)-2-propenamide (45). To a solution of 3-hydroxyphenylenediamine (**44**) (250 mg, 2.01 mmol) in DMF (5 mL) was added 60% NaH in oil (84.6 mg, 2.11 mmol) with cooling in an ice/water bath under a nitrogen atmosphere. After 20 min, **16b** (993 mg, 2.11 mmol) was added to the reaction mixture. The reaction mixture was stirred at 0 °C for 30 min and at ambient temperature for 2 h, then poured into water, and the precipitate was collected by vacuum filtration. The precipitate was purified by flash silica gel chromatography ($CHCl_3$/MeOH, 20:1) to afford **45** (748 mg, 66.6%) as a brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.22 (s, 3H), 3.28 (s, 3H), 3.50 (br s, 4H), 3.69 (dd, J = 17, 4 Hz, 1H), 3.94 (dd, J = 17, 5 Hz, 1H), 5.34 (s, 2H), 6.40−6.50 (m, 2H), 6.60−6.76 (m, 3H), 7.32 (d, J = 7.5 Hz, 1H), 7.50 (d, J = 7.5 Hz, 1H), 7.53 (d, J = 15 Hz, 1H), 7.85 (dd, J = 7.5, 2 Hz, 1H), 7.27−7.36 (m, 4H), 7.49 (d, J = 7.5 Hz, 1H), 7.51 (d, J = 15 Hz, 1H), 7.84 (br d, J = 7.5 Hz, 1H), 8.09 (br s, 1H), 8.22 (br d, J = 7.5 Hz, 1H), 8.36 (br s, 1H); MS (ESI) *m*/*z* 557 (M + 1). Anal. ($C_{26}H_{26}Cl_2N_6O_4$) C, H, N.

(2*E*)-3-[6-(Acetylamino)-3-pyridinyl]-*N*-[2-(2,4-dichloro-3-{[(2-methoxy-1*H*-benzimidazol-7-yl)oxy]methyl}methylanilino)-2-oxoethyl]-2-propenamide (46). To a solution of **45** (735 mg, 1.32 mmol) in AcOH (7.4 mL) was added tetramethyl orthocarbonate (270 mg, 1.98 mmol), and the mixture was stirred at ambient temperature for 5 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in $CHCl_3$. The organic layer 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 ($CHCl_3$/MeOH, 30:1) to afford **46** (654 mg, 83.0%) as a pale-brown solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.21 (s, 3H), 3.29 (s, 3H), 3.59 (br d, J = 17 Hz, 1H), 4.10−4.22 (m, 4H), 5.30 (d, J = 10 Hz, 1H), 5.59 (d, J = 10 Hz, 1H), 6.48 (d, J = 15 Hz, 1H), 6.78 (br s, 1H), 6.83 (d, J = 7.5 Hz, 1H), 7.12 (dd, J = 7.5, 7.5 Hz, 1H), 7.20−7.29 (m, 1H), 7.32 (d, J = 7.5 Hz, 1H), 7.49 (d, J = 7.5 Hz, 1H), 7.65 (d, J = 15 Hz, 1H), 7.85 (br d, J = 7.5 Hz, 1H), 8.09 (br s, 1H), 8.23 (br d, J = 7.5 Hz, 1H), 8.37 (br s, 1H); MS (ESI) *m*/*z* 597 (M + 1). Anal. ($C_{28}H_{26}Cl_2N_6O_5$) C, H, N.

Ethyl {4-[(3-{[({(2*E*)-3-[6-(Acetylamino)-3-pyridinyl]-2-propenoyl}amino)acetyl](methyl)amino}-2,6-dichlorobenzyl)oxy]-2-methoxy-1*H*-benzimidazol-1-yl}acetate (47a). To a solution of **46** (100 mg, 0.168 mmol) in DMF (1.5 mL) were added ethyl bromoacetate (28.7 mg, 0.172 mmol) and K_2CO_3 (69.5 mg, 0.503 mmol) at ambient temperature, and the mixture was stirred at the same temperature for 15 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 purified by preparative thin-layer chromatography ($CHCl_3$/MeOH, 10:1) to afford **47a** (51.1 mg, 44.5%) as a colorless amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27 (t, J = 7 Hz, 3H), 2.20 (s, 3H), 3.28 (s, 3H), 3.65 (dd, J = 17, 4 Hz, 1H), 3.95 (dd, J = 17, 4 Hz, 1H), 4.20 (s, 2H), 4.24 (q, J = 7 Hz, 2H), 4.68 (s, 2H), 5.66 (s, 2H), 6.47 (d, J = 15 Hz, 1H), 6.70 (br s, 1H), 6.77 (d, J = 8 Hz, 1H), 6.86 (d, J = 8 Hz, 1H), 7.10 (dd, J = 8, 8 Hz, 1H), 7.30 (d, J = 8 Hz, 1H), 7.48−7.56 (m, 2H), 7.83 (dd, J = 8, 2 Hz, 1H), 8.15−8.22 (m, 2H), 8.35 (d, J = 2 Hz, 1H); MS (ESI) *m*/*z* 683 (M + 1). Anal. ($C_{32}H_{32}Cl_2N_6O_7$) C, H, N.

Compounds **47b** and **47c** were prepared following the procedure described above for **47a**.

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[32] 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 a Polytron. The homogenate was centrifuged to remove cellular debris (1000*g*, 20 min, 4 °C), and the supernatant was centrifuged (100000*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 [^{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 are transfected with, and stably express human B_2 receptors, have been described previously.[19] 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 (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.

Agonist-Induced Inositol Phosphate Production. Inositol phosphate formation was measured essentially as described previously.[19] CHO cells expressing the human B_2 receptor were seeded in 12-well plates at a 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.[33] 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).

Agonist-Induced PGE_2 Production. Human fibroblasts (WI-38, ATCC CCL75) were grown in Eagle's essential medium (E-MEM) with 10% fetal bovine serum (FBS). The fibroblasts were seeded in 24-well plates at a density of 1×10^5 cells/well with 0.5 mL of E-MEM including 1% FBS and were cultured for 24 h at 37 °C. Then the medium in the culture was removed by aspiration, and Hanks' balanced salt solution containing 0.1% BSA (Hanks-BSA buffer) was added. After the Hanks-BSA buffer was removed by aspiration, different concentrations of a test compound in Hanks-BSA buffer was added , and the cells were incubated for 30 min at 37 °C. After the incubation, the supernatant was collected, and the PGE_2 level included therein was measured by a PGE_2 EIA kit (Cayman Chemical Co.). The agonist-induced PGE_2 production was calculated by subtracting the control PGE_2 production determined in the absence of the compound. The efficacy of the compound was expressed as the relative agonistic activity in PGE_2 production compared to that of BK (100 nM).

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

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Supporting Information Available: Physical data for **9c**−**f**, **17b**−**e**, **18**, **19a**,**b**, **22b**−**e**, **23**, **24**, **29**−**33**, **35**−**38**, **42**, **47b**, and **47c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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