A Novel Class of Orally Active Non-Peptide Bradykinin B_2 Receptor Antagonists. 2. Overcoming the Species Difference between Guinea Pig and Man

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Recently we reported the identification of a series of 8-[[3-(*N*-acylglycyl-*N*-methylamino)-2,6-dichlorobenzyl]oxy]-3-halo-2-methylimidazo[1,2-*a*]pyridines as the first orally active non-peptide bradykinin (BK) B_2 receptor antagonists (**1**−**3**). These compounds inhibited the specific binding of [^{3}H]BK to guinea pig ileum membrane preparations expressing B_2 receptors with nanomolar IC_{50}'s and also displayed in vivo functional antagonistic activities against BK-induced bronchoconstriction in guinea pigs at 1 mg/kg by oral administration. However, it was found that their affinities for the B_2 receptors in human A-431 cells (human epidermoid carcinoma) were much lower. Intensive modifications of the terminal substituents at the glycine moiety elucidated the structure−activity relationships (SAR) for human B_2 receptors, leading to an extended basic framework which incorporated a novel key pharmacophore. Thus, we overcame the species difference and identified the first clinical candidate **18c** (FR167344) with IC_{50}'s of 0.66 and 1.4 nM for guinea pig ileum and human A-431 cells, respectively. This compound displayed in vivo functional antagonistic activity against BK-induced bronchoconstriction in guinea pigs with an ED_{50} value of 0.17 mg/kg by oral administration. This novel non-peptide B_2 antagonist is extremely potent both in vitro and in vivo by oral administration and is expected to be the first member of a new class of drug for the treatment of various inflammatory diseases.

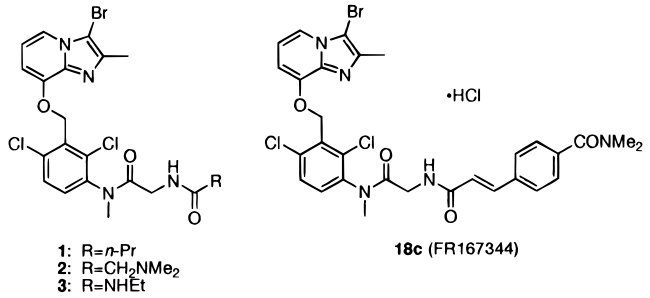
Introduction

Bradykinin (BK) is an endogenous nonapeptide generated by tissue and plasma kallikreins. Because of its highly potent proinflammatory activities, BK has been implicated in a variety of pathophysiological responses, including pain, inflammation, asthma, rhinitis, and hypotension.[1−8] Two types of BK receptors, designated as B_1 and B_2, have been identified by molecular cloning and pharmacological means.[1,4,9−11] B_2 receptors are expressed constitutively in many tissues and are thought to mediate most of the biological actions of BK.[1,9]

The search for antagonists of the B_2 receptor has been pursued for many years. A number of peptide BK B_2 antagonists have been developed,[12−16] including the clinically evaluated second-generation antagonists Icatibant (Hoe140)[12,13] and Bradycor (CP0127).[14] Despite their highly potent B_2 antagonistic activity, their therapeutic use is still limited because of their peptidic nature. On the other hand, only very few non-peptide antagonists have been disclosed,[17−19] and until our earlier report[20] there had been no description of potent orally active non-peptide B_2 antagonists.

Recently we reported the discovery of the first orally active non-peptide BK B_2 antagonists incorporating an 8-[[3-(*N*-acylglycyl-*N*-methylamino)-2,6-dichlorobenzyl]-oxy]-3-halo-2-methylimidazo[1,2-*a*]pyridine skeleton as the basic framework.[20] The representative lead compounds **1**−**3** (Chart 1) inhibited the specific binding of [^{3}H]BK to guinea pig ileum membrane preparations expressing B_2 receptors with nanomolar IC_{50}'s and also displayed in vivo functional antagonistic activities against BK-induced bronchoconstriction in guinea pigs by oral administration with ED_{50} values of <1 mg/kg. However, it was found that these small non-peptide antagonists bound to the B_2 receptors in human A-431 cells rather weakly. The structure−activity relationships (SAR) of the *N*-substituents of the glycine moiety suggested that this group plays a key role in the interaction with human A-431 B_2 receptors and that an additional pharmacophore might be necessary for stronger binding. Intensive chemical modifications of this terminal group enabled us to overcome the species difference without loss of the potent oral in vivo antagonistic activity leading to our first clinical candi-

Chart 1



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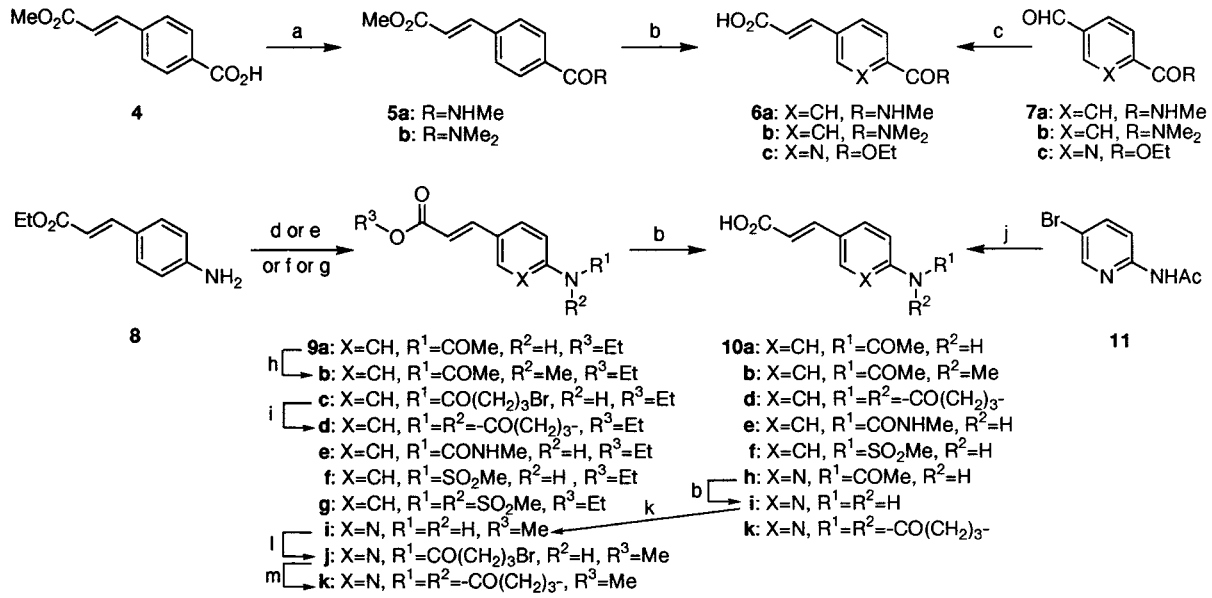
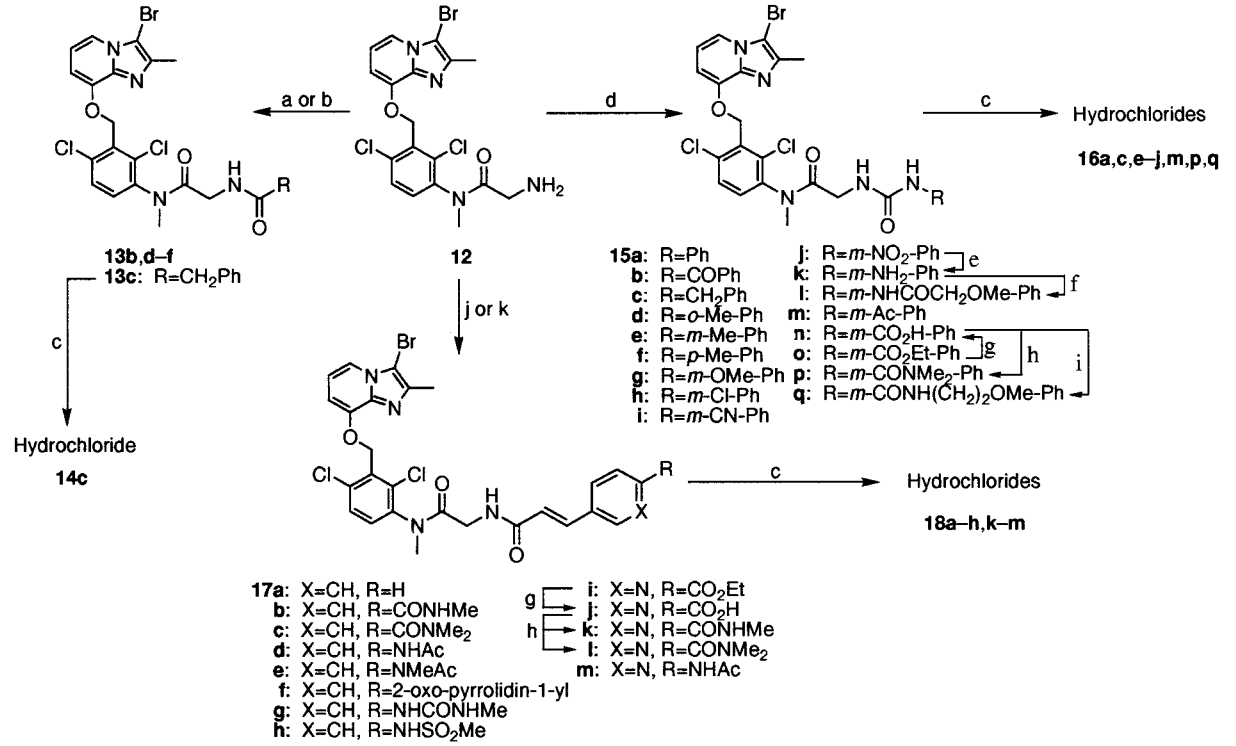
Scheme 1[a]



[a] (a) RH·HCl, WSCD, HOBt, DMF; (b) 1 N NaOH, EtOH or MeOH; (c) $CH_2(CO_2H)_2$, pyridine, EtOH; (d) Ac_2O, CH_2Cl_2; (e) $Br(CH_2)_3CO_2H$, WSCD·HCl, HOBt, DMF; (f) MeNCO, CH_2Cl_2; (g) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (h) MeI, NaH, DMF; (i) K_2CO_3, DMF; (j) acrylic acid, $Pd(OAc)_2$, PPh_3, *n*-Bu_3N, xylene; (k) $SOCl_2$, MeOH; (l) $Br(CH_2)_3COCl$, Et_3N, CH_2Cl_2; (m) NaH, DMF.

Scheme 2[a]



[a] (a) RCOCl, Et_3N, CH_2Cl_2; (b) $Ph(CH_2)_3CO_2H$, WSCD·HCl, HOBt, DMF; (c) HCl–MeOH; (d) RNCO, CH_2Cl_2; (e) $SnCl_2$, EtOH; (f) $MeOCH_2CO_2H$, WSCD·HCl, HOBt, DMF; (g) 1 N NaOH, EtOH; (h) Me_2NH·HCl or $MeNH_2$·HCl, WSCD, HOBt, DMF; (i) $MeO(CH_2)_2NH_2$, WSCD·HCl, HOBt, DMF; (j) cinnamoyl chloride, Et_3N, CH_2Cl_2; (k) **6a**–**c** or **10a**–**f**,**h**,**k**, WSCD·HCl, HOBt, DMF.

date **18c** (FR167344).[21] Herein we wish to describe the SAR culminating in the discovery of **18c**.

Chemistry

The compounds described in this study are shown in Tables 1–3, and their synthetic methods are outlined in Schemes 1 and 2. Preparation of (*E*)-4-(substituted)-cinnamic acids **6a**,**b** and **10a**–**f** and (*E*)-3-[6-(substituted)pyridin-3-yl]acrylic acids **6c** and **10h**,**i**,**k** are shown in Scheme 1. Condensation of methyl (*E*)-4-carboxycinnamate (**4**), which was synthesized by Wittig reaction of terephthalaldehydic acid and methyl (triphenylphosphoranylidene)acetate, with the corresponding amines in the presence of 1-ethyl-3-[(dimethylamino)propyl]-carbodiimide (WSCD) and 1-hydroxybenzotriazole (HOBt) followed by alkaline hydrolysis gave the acids **6a**,**b**. Acetylation of ethyl (*E*)-4-aminocinnamate (**8**) and subsequent alkylation of the acetamide **9a** with methyl

iodide gave the *N*-methylacetamide **9b**. The acids **10a**,**b** were obtained by saponification of the esters **9a**,**b**. Coupling of **8** with 4-bromobutyric acid followed by intramolecular cyclization of **9c** in the presence of potassium carbonate in DMF provided pyrrolidone **9d** which was hydrolyzed to **10d**. Reaction of the amine **8** with methyl isocyanate followed by hydrolysis gave the acid **10e**. Sulfonylation of **8** with methanesulfonyl chloride gave a mixture of mono- and disulfonamides **9f**,**g**, which were hydrolyzed to yield the acid **10f**. Two types of alternative reactions were performed to prepare the acids. The first was Perkin reaction of the aldehydes **7a**−**c** with malonic acid in the presence of pyridine in EtOH to give the acids **6a**−**c**. The second was Heck reaction of the bromopyridine **11**[22] with acrylic acid to yield the acid **10h**. Removal of the acetyl group of **10h** and successive esterification with $SOCl_2$ in MeOH gave the amino ester **9i**. Coupling of **9i** with 4-bromobutyryl chloride and intramolecular cyclization by sodium hydride followed by hydrolysis provided the pyrrolidone derivative **10k**.

Modifications of the substituent at the amino group of **12**[20] are shown in Scheme 2. Acylation of **12** gave the amides **13b**−**f**. Compound **13c** was treated with 10% hydrogen chloride in MeOH to afford the hydrochloride **14c**. Reaction of **12** with various isocyanates yielded the corresponding ureas **15a**−**j**,**m**,**o**. The nitro group of **15j** was reduced with $SnCl_2$ in EtOH followed by coupling with methoxyacetyl chloride to give the amide **15l**. Hydrolysis of the ester **15o** and subsequent coupling with appropriate amines afforded the carbamoyls **15p**,**q**. The ureas **15a**,**c**,**e**−**j**,**m**,**p**,**q** were converted to the corresponding hydrochlorides **16a**,**c**,**e**−**j**,**m**,**p**,**q**, respectively. Acylation of **12** with cinnamoyl chloride gave the cinnamamide **17a**. The acrylamide derivatives **17b**−**i**,**m** were synthesized by coupling of **12** with the acids **6a**−**c** and **10a**−**f**,**g**,**k** in the presence of WSCD·HCl and HOBt in DMF. Saponification of the ester **17i** followed by coupling with amines yielded the carbamoyls **17k**,**l**. The hydrochlorides **18a**−**h**,**k**−**m** were also obtained by treatment of **17a**−**h**,**k**−**m** with 10% hydrogen chloride in MeOH.

Pharmacology

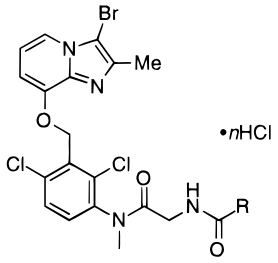
All compounds were tested for inhibition of the specific binding of [^{3}H]BK to B_2 receptors in guinea pig ileum membrane preparations as previously reported,[20,21] and they were also evaluated for inhibition of the specific binding of [^{3}H]BK to B_2 receptors in human A-431 cells. Compounds having potent binding affinities were then tested for in vivo functional antagonistic activity in inhibiting BK-induced bronchoconstriction in guinea pigs by oral administration.[20,21a]

Results and Discussion

Recently we reported on the first series of orally active non-peptide BK B_2 receptor antagonists incorporating an 8-[[3-(*N*-acylglycyl-*N*-methylamino)-2,6-dichlorobenzyl]oxy]-3-halo-2-methylimidazo[1,2-*a*]pyridine skeleton as the basic framework.[20] The representative lead compounds **1**−**3** inhibited the specific binding of [^{3}H]-BK to guinea pig ileum membrane preparations with nanomolar IC_{50}'s and also displayed dose-dependent in vivo functional antagonistic activities against BK-induced bronchoconstriction in guinea pigs at 1 mg/kg and above by oral administration (Table 1). Considering the possibility of clinical studies, these compounds were then advanced for evaluation of binding activity to human B_2 receptors in A-431 cells.[23] Despite using a 20-fold higher concentration of [^{3}H]BK in the human A-431 cell system than in guinea pig ileum membrane preparations, a representative second-generation peptide B_2 antagonist, Icatibant, potently inhibited the specific binding with an IC_{50} value of 3.3 nM, ca. 37-fold higher than that in guinea pigs. However, it was revealed that our non-peptide B_2 antagonists showed only weak binding affinities in the human A-431 cell system. The IC_{50} values of **1**, **2**, and **3** for the B_2 receptors of human A-431 cells were ca. 70-, 350-, and 330-fold higher than those in guinea pigs, respectively. To overcome this species difference, we initially investigated the SAR of the terminal *N*-acyl substituents of the glycine moiety (Table 1). Replacement of the terminal methyl group of **13a** with a *n*-propyl (**1**) or *n*-pentyl (**13b**) group increased their B_2 binding affinities in both the human A-431 cell and the guinea pig ileum systems. Introduction of the phenyl group (**14c**) to **13a** also increased both affinities to the same level of that of **1**, indicating that a bulky substituent at the glycine nitrogen is tolerated in the binding site of both B_2 receptors. Extending the methylene of **14c** to an ethylene (**13d**) afforded a 2-fold increase in binding affinity in the human A-431 cell system, while further extension to the propylene (**13e**) resulted in a slight loss of activity. Furthermore, the cinnamamide **18a** resulted in a ca. 2- and 3-fold improvement of the B_2 binding affinities in guinea pig ileum and human A-431 cell systems with IC_{50}'s of 0.78 and 120 nM, respectively. On the other hand, as shown by **2** and **13f**, introduction of heteroatoms to the terminal chain failed to improve affinities for the human receptor. It was interesting that in the benzoylurea (**15b**), binding affinity in the guinea pig system decreased much more severely than in the human system compared with the phenylurea (**16a**). In the urea series, **16a** exhibited more potent B_2 binding affinities than ethylurea (**3**) and benzylurea (**16c**) in both systems. These results suggested that a large planar terminal substituent, incorporating an aromatic ring such as a cinnamamide or phenylurea moiety, is favorable for interaction with both B_2 receptors. However, neither **18a** nor **16a** showed significant antagonistic activities in vivo by oral administration. Therefore we investigated the effects of substituents on the terminal phenyl ring of **18a** and **16a** to improve both binding affinity for human A-431 B_2 receptors and in vivo antagonistic activity by oral administration.

Introduction of substituents to the terminal phenyl ring of the phenylurea (**16a**) is summarized in Table 2. Comparison of the B_2 binding affinities of **15d** and **16e**,**f** indicated that ortho and para substitutions at the phenyl ring were not sterically tolerated in guinea pigs, while ortho substitution retained and para substitution only slightly decreased the activities in human A-431 cells. According to these results, we then introduced various substituents at the 3-position of the phenyl ring, where methyl substitution was tolerated in both guinea pig ileum and human A-431 cell systems. Replacement

Table 1. Introduction of Acyl Substituents at the Glycine Moiety



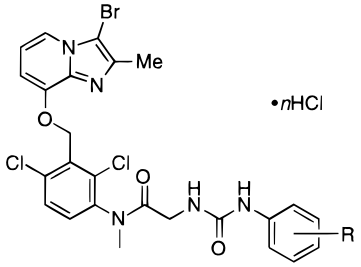
compd	R	*n*	in vitro IC_{50} (nM) guinea pig ileum[a]	in vitro IC_{50} (nM) human A-431 cell[b]	in vivo % inhibn (po)[c] 1 mg/kg	in vivo % inhibn (po)[c] 3.2 mg/kg	synth method[d]	mp (°C)	formula[e]
13a	Me	1	16	4100	62.3 ± 15.6**	94.2 ± 2.2***	ref 20	179−180	$C_{20}H_{19}BrCl_2N_4O_3$·HCl
1	*n*-Pr	0	8.9	610	52.7 ± 14.6	NT[f]	ref 20	158−159	$C_{22}H_{23}BrCl_2N_4O_3$
13b	*n*-Pen	0	5.6	240	NT	NT	B	131−132	$C_{24}H_{27}BrCl_2N_4O_3$
2	CH_2NMe_2	0	2.4	830	72.4 ± 7.8**	92.8 ± 2.4***	ref 20	175−177	$C_{22}H_{24}BrCl_2N_5O_3$
14c	CH_2Ph	1	5.3	670	NT	19.8 ± 10.3	D	amorphous	$C_{26}H_{23}BrCl_2N_4O_3$·HCl
13d	$(CH_2)_2Ph$	0	1.4	350	NT	NT	B	amorphous	$C_{27}H_{25}BrCl_2N_4O_3$
13e	$(CH_2)_3Ph$	0	8.9	480	NT	NT	C	amorphous	$C_{28}H_{27}BrCl_2N_4O_3$
13f	CH_2OPh	0	4.2	510	NT	NT	B	amorphous	$C_{26}H_{23}BrCl_2N_4O_4$
18a	(*E*)-CH=CHPh	1	0.78	120	0 ± 19.2	NT	D	amorphous	$C_{27}H_{23}BrCl_2N_4O_3$·HCl
3	NHEt	0	9.0	3000	57.2 ± 4.9***	78.1 ± 6.2***	ref 20	171−173	$C_{21}H_{22}BrCl_2N_5O_3$
16a	NHPh	1	1.4	150	NT	30.7 ± 11.1	D	amorphous	$C_{25}H_{22}BrCl_2N_5O_3$·HCl
15b	NHCOPh	0	340	510	NT	NT	A	187−190	$C_{26}H_{22}BrCl_2N_5O_4$
16c	$NHCH_2Ph$	1	11	570	NT	24.1 ± 9.8	D	amorphous	$C_{26}H_{24}BrCl_2N_5O_3$·HCl
Icatibant			0.09	3.3	NT	NT			

[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 (1.2 nM) to A-431 cells (human epidermoid carcinoma) which express B_2 receptors 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] 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[27] as previously reported. After 5 min, compounds were orally administered. After 30 min, BK was administered again and bronchoconstriction was measured. Percent inhibition was calculated from the values of percent responses of drug-treated and control 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] For details, see the Experimental Section. [e] Analyses for C, H, and N were within ±0.4% of the expected value for the formula. [f] NT, not tested.

of the 3-methyl group of **16e** with a chloro group (**16h**) decreased the affinity in human A-431 cells selectively, while amino (**15k**) and carboxylic acid (**15n**) resulted in loss of affinity in both systems. A 3-methoxy (**16g**), cyano (**16i**), nitro (**16j**), or ethoxycarbonyl (**15o**) group retained activities. On the other hand, 3-methoxyacetamide (**15l**) and 3-acetyl (**16m**) derivatives were twice as potent as **16a** in human A-431 cells. Furthermore, 3-carbamoyl derivatives (**16p**,**q**) afforded a 2-fold and 1 order of magnitude increase in B_2 binding affinities in guinea pigs and human A-431 cells, respectively. These results suggest that the carbonyl oxygen of the carbamoyl, amide, and ketone groups may have electrostatic interaction with the B_2 receptor. However, these compounds failed to display significant in vivo antagonistic activities at 1 mg/kg by oral administration.

With these results in mind, we investigated substitutions with carbamoyl, amide, and related functional groups at the 4-position of the phenyl ring of the cinnamamide **18a**, which might correspond to the 3-position of the phenylurea with respect to the topology (Table 3). Later, direct comparison of 3- and 4-(methylcarbamoyl)cinnamamide derivatives was carried out in another series of B_2 antagonists. These results which support our hypothesis will be reported in due course.[24] Introduction of the *N*-methylcarbamoyl (**18b**) and the *N*,*N*-dimethylcarbamoyl (**18c**) groups increased B_2 binding affinities in human A-431 cells by approximately 2 orders of magnitude, while retaining the affinities in guinea pig ileum. Furthermore, these compounds also significantly inhibited BK-induced bronchoconstriction at 1 mg/kg by oral administration. In particular, compound **18c** was quite potent even at the lower dose, 0.32 mg/kg, with an ED_{50} value of 0.17 mg/kg po. In this series, an acetamide group afforded the same degree of increase in human A-431 cell B_2 affinity, resulting in **18d**. *N*-Methylation of the acetamide group of **18d** gave **18e** which was 10- and 2-fold less potent in human A-431 cells and guinea pigs, respectively. Although **18d** failed to show significant in vivo antagonistic activity at 1 mg/kg po, its *N*-methyl derivative, **18e**, exhibited 84.3% inhibition at the same oral dose. Cyclization of the *N*-methylamide group to the pyrrolidone moiety (**18f**) regained the binding activity in both systems and preserved the potent in vivo activity. In addition, not only the carbamoyl (**18b**,**c**) and the amide (**18d**,**f**) but also the urea (**18g**) and the sulfonamide (**18h**) derivatives displayed potent binding activities in human A-431 cells, whereas they exhibited only weak in vivo activity. These results suggest that the oxygen atom at the terminal carbonyl or the sulfonyl group of the cinnamamide series may interact with the binding site of the B_2 receptor in human A-431 cells more efficiently, maybe as a hydrogen bond acceptor, than that of the phenylurea derivatives. Further investiga-

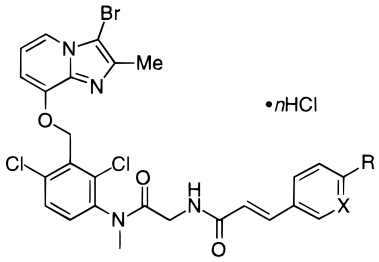
Table 2. Introduction of Substituents to the Phenylurea Moiety



compd	R	*n*	in vitro IC_{50} (nM) guinea pig ileum[a]	in vitro IC_{50} (nM) human A-431 cell[b]	in vivo % inhibn[c] 1 mg/kg, po	synth method[d]	mp (°C)	formula[e]
16a	H	1	1.4	150	30.7 ± 11.1	D	amorphous	$C_{25}H_{22}BrCl_2N_5O_3$·HCl
15d	*o*-Me	0	83	150	NT[f]	A	amorphous	$C_{26}H_{24}BrCl_2N_5O_3$
16e	*m*-Me	1	2.7	140	27.4 ± 14.5[g]	D	amorphous	$C_{26}H_{24}BrCl_2N_5O_3$·HCl
16f	*p*-Me	1	12	260	35.0 ± 18.6[g]	D	amorphous	$C_{26}H_{24}BrCl_2N_5O_3$·HCl
16g	*m*-OMe	1	2.3	200	0 ± 9.0	D	amorphous	$C_{26}H_{24}BrCl_2N_5O_4$·HCl
16h	*m*-Cl	1	3.8	1900	14.5 ± 8.4	D	amorphous	$C_{25}H_{21}BrCl_3N_5O_3$·HCl
16i	*m*-CN	1	1.4	190	4.7 ± 13.5	D	amorphous	$C_{26}H_{21}BrCl_2N_6O_3$·HCl
16j	*m*-NO_2	1	4.2	180	13.6 ± 15.7	D	amorphous	$C_{25}H_{21}BrCl_2N_6O_5$·HCl
15k	*m*-NH_2	0	15	>1000	NT	Ex[h]	amorphous	$C_{25}H_{23}BrCl_2N_6O_3$
15l	*m*-$NHCOCH_2OMe$	0	2.7	75	NT	C	238−239	$C_{28}H_{27}BrCl_2N_6O_5$
16m	*m*-Ac	1	0.79	61	24.9 ± 6.3*	D	amorphous	$C_{27}H_{24}BrCl_2N_5O_4$·HCl
15n	*m*-COOH	0	63	5200	NT	E	248−250	$C_{26}H_{22}BrCl_2N_5O_5$
15o	*m*-COOEt	0	2.2	150	NT	A	amorphous	$C_{28}H_{26}BrCl_2N_5O_5$
16p	*m*-$CONMe_2$	1	0.76	20	23.2 ± 13.0	D	amorphous	$C_{28}H_{27}BrCl_2N_6O_4$·HCl
16q	*m*-$CONH(CH_2)_2OMe$	1	0.79	20	16.1 ± 6.9	D	amorphous	$C_{29}H_{29}BrCl_2N_6O_5$·HCl

[a−f] See corresponding footnotes in Table 1. [g] Percent inhibition at a doses of 3.2 mg/kg. [h] Ex, experimental procedure described.

Table 3. Introduction of Substituents to the Cinnamamide Moiety



compd	R	X	*n*	in vitro IC_{50} (nM) guinea pig ileum[a]	in vitro IC_{50} (nM) human A-431 cell[b]	in vivo % inhibn (po)[c] 0.32 mg/kg	in vivo % inhibn (po)[c] 1 mg/kg	synth method[d]	mp (°C)	formula[e]
18a	H	CH	1	0.78	120	NT[f]	0 ± 19.2	D	amorphous	$C_{27}H_{23}BrCl_2N_4O_3$·HCl
18b	CONHMe	CH	1	0.50	1.5	NT	53.2 ± 3.6***	D	amorphous	$C_{29}H_{26}BrCl_2N_5O_4$·HCl
18c	$CONMe_2$	CH	1	0.66	1.4	74.8 ± 2.9*	86.7 ± 4.9**	D	143−146	$C_{30}H_{28}BrCl_2N_5O_4$·HCl·$2H_2O$
18d	NHAc	CH	1	0.55	1.6	NT	23.1 ± 10.1	D	amorphous	$C_{29}H_{26}BrCl_2N_5O_4$·HCl
18e	NMeAc	CH	1	1.1	16	NT	84.3 ± 6.1**	D	amorphous	$C_{30}H_{28}BrCl_2N_5O_4$·HCl
18f		CH	1	0.59	4.1	20.7 ± 2.0	82.0 ± 6.1***	D	amorphous	$C_{31}H_{28}BrCl_2N_5O_4$·HCl
18g	NHCONHMe	CH	1	0.89	2.6	NT	18.8 ± 5.7*	D	amorphous	$C_{29}H_{27}BrCl_2N_6O_4$·HCl
18h	$NHSO_2Me$	CH	1	3.9	4.8	NT	0 ± 16.1	D	amorphous	$C_{28}H_{26}BrCl_2N_5O_5S$·HCl
18k	CONHMe	N	2	0.36	9.1	NT	89.4 ± 4.1**	D	amorphous	$C_{28}H_{25}BrCl_2N_6O_4$·2HCl
18l	$CONMe_2$	N	2	0.44	3.7	NT	92.4 ± 5.3**	D	amorphous	$C_{29}H_{27}BrCl_2N_6O_4$·2HCl
18m	NHAc	N	2	0.38	2.4	74.6 ± 8.4**	76.4 ± 9.9*	D	amorphous	$C_{28}H_{25}BrCl_2N_6O_4$·2HCl

[a−f] See corresponding footnotes in Table 1.

tions on this key pharmacophore will be discussed in due course.[24]

Next we investigated replacement of the terminal phenyl ring with a 3-pyridyl group, whose nitrogen atom might correspond to the 3-substituent of the phenylurea in terms of topology. This modification caused a slight loss of binding affinities in human A-431 cells. However, it is noteworthy that **18k**,**m** displayed a remarkable improvement in in vivo activity compared to the corresponding cinnamamides **18b**,**d**. Thus, we have successfully overcome the species difference in affinity between BK B_2 receptors in guinea pigs and humans by extending the basic framework leading to cinnamamide derivatives incorporating a novel pharmacophore. On the basis of the most potent binding affinity for human B_2 receptors and the excellent in vivo antago-

nistic activities, compound **18c** has been identified as a candidate for clinical development. Further pharmacological characterization of **18c** revealed its potent, selective,[21a,b] and insurmountable antagonistic activity for cloned human B_2 receptors.[21b]

Conclusion

We found that our first orally active non-peptide BK B_2 antagonists **1**−**3** were much less potent in the human A-431 cell system than in guinea pigs. To overcome this species difference, we investigated the SAR of the terminal substituents on the glycine moiety. Intensive chemical modifications revealed that the (*E*)-cinnamoyl moiety with the carbamoyl or the amide groups at the 4-position or the (*E*)-3-(pyridin-3-yl)acryloyl moiety with the carbamoyl or the amide groups at the 6-position of the pyridine ring significantly enhanced the binding affinity in human assay systems, leading to the discovery of the first clinical candidate **18c**. Since **18c** is selective, highly potent, and orally active in various inflammatory models,[21c] it is expected to be the first of a new class of drugs for the treatment of various inflammatory diseases.

Experimental Section

Chemistry. Melting points were determined on a Mel-Temp (Mitamura Riken Kogyo, Japan) and are uncorrected. The 200-MHz proton NMR spectra were recorded on a Brucker AM200 or Varian Gemini 300 spectrometer, and shifts are expressed in δ (ppm) with TMS as internal standard. Mass spectra were recorded on a VG (Fisons) ZAB-SE (FAB) or Micromass Platform (ESI). IR spectra were taken with a Perkin-Elmer FTIR 1600 spectrometer in Nujol or KBr and are expressed in cm^{-1}. 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). Extraction solvents were dried over magnesium sulfate.

Methyl (*E*)-4-Carboxycinnamate (4). To a suspension of phthalaldehydic acid (1.00 g, 6.66 mmol) in dry THF (15 mL) was added methyl (triphenylphosphoranylidene)acetate (2.50 g, 7.33 mmol) in an ice−water bath under nitrogen. The reaction mixture was stirred at the same temperature for 30 min and then stirred at ambient temperature. After 1 h, the reaction mixture was poured into saturated aqueous sodium bicarbonate solution and washed with AcOEt twice. The aqueous layer was adjusted to pH 3 with 1 N HCl. The precipitated solid was collected by vacuum filtration, washed with water, and dried in vacuo. The solid was crystallized from MeCN to give **4** (1.21 g, 88.4%) as colorless crystals: mp 242−243 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.74 (3H, s), 6.76 (1H, d, J = 16 Hz), 7.73 (1H, d, J = 16 Hz), 7.85 (2H, d, J = 8 Hz), 7.96 (2H, d, J = 8 Hz). Anal. ($C_{11}H_{10}O_4$) C, H.

4-(*N*-Methylcarbamoyl)benzaldehyde (7a). To a suspension of terephthalaldehydic acid (3.00 g, 20.0 mmol) in dry CH_2Cl_2 (6 mL) were added thionyl chloride (3.63 g, 24 mmol) and DMF (0.5 mL) dropwise at ambient temperature under nitrogen. The reaction mixture was refluxed for 2 h. After cooling, this mixture was added to 40% methylamine−H_2O (6.2 mL, 72 mmol) dropwise over 15 min in an ice−water bath, and the reaction mixture stirred for 1 h at the same temperature. The solid that precipitated was collected by vacuum filtration, washed with water and hexane, and dried. The solid was recrystallized from AcOEt−hexane to give **7a** (2.08 g, 63.7%) as colorless crystals: mp 160−161 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.97 (3H, d, J = 6 Hz), 7.97 (2H, d, J = 9 Hz), 8.02 (2H, d, J = 9 Hz), 8.67 (1H, m), 10.06 (1H, s). Anal. ($C_9H_9NO_2$) C, H, N.

Compound **7b** was prepared following a procedure similar to the preparation of **7a**.

(*E*)-3-[6-(Ethoxycarbonyl)pyridin-3-yl]acrylic Acid (6c). A mixture of **7c**[25] (2.14 g, 13.5 mmol) and malonic acid (1.54 g, 14.8 mmol) in dry pyridine (1.1 mL) and EtOH (3.2 mL) was refluxed for 2 h. After the mixture was cooled, the solid that precipitated was collected by vacuum filtration, washed with AcOEt, and dried. The solid was crystallized from MeCN to give **6c** (2.04 g, 68.7%) as colorless crystals: mp 202−204 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 1.33 (3H, t, J = 7 Hz), 4.36 (2H, q, J = 7 Hz), 6.80 (1H, d, J = 16 Hz), 7.69 (1H, d, J = 16 Hz), 8.07 (1H, d, J = 9 Hz), 8.33 (1H, dd, J = 9, 2 Hz), 9.00 (1H, d, J = 2 Hz). Anal. ($C_{11}H_{11}NO_4$) C, H, N.

Compounds **6a**,**b** were prepared following a procedure similar to the preparation of **6c**.

Ethyl (*E*)-4-Acetamidocinnamate (9a). A mixture of **8** (2.00 g, 10.5 mmol) and acetic anhydride (1.28 g, 12.6 mmol) in dry CH_2Cl_2 (20 mL) was stirred for 1 h. The reaction mixture was evaporated in vacuo. The residue was partitioned with CH_2Cl_2 and saturated aqueous sodium bicarbonate solution. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was then crystallized from isopropyl ether to give **9a** (2.35 g, 96.3%) as colorless crystals: mp 134−136 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.32 (3H, t, J = 7 Hz), 2.20 (3H, s), 4.26 (2H, q, J = 7 Hz), 6.37 (1H, d, J = 16 Hz), 7.36 (1H, br s), 7.49 (2H, d, J = 8 Hz), 7.55 (2H, d, J = 8 Hz), 7.63 (1H, d, J = 16 Hz). Anal. ($C_{13}H_{15}NO_3$) C, H, N.

Ethyl (*E*)-4-(*N*-Methylacetamido)cinnamate (9b). To a solution of **9a** (1.2 g, 5.15 mmol) in dry DMF (12 mL) was added portionwise 60% sodium hydride in oil (226 mg, 5.66 mmol) in an ice−water bath under nitrogen. After 30 min, methyl iodide (803 mg, 5.66 mmol) was added therein, and the reaction mixture was stirred at ambient temperature for 30 min. The mixture was poured into water and extracted with AcOEt twice. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was recrystallized from isopropyl ether to give **9b** (979 mg, 76.9%) as colorless crystals: mp 74−75 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.35 (3H, t, J = 7 Hz), 1.93 (3H, br s), 3.29 (3H, s), 4.29 (2H, q, J = 7 Hz), 6.44 (1H, d, J = 16 Hz), 7.21 (2H, d, J = 8 Hz), 7.58 (2H, d, J = 8 Hz), 7.69 (1H, d, J = 16 Hz). Anal. ($C_{14}H_{17}NO_3$) C, H, N.

Ethyl (*E*)-4-(2-Oxopyrrolidin-1-yl)cinnamate (9d). To a stirred solution of **9c** (420 mg, 1.23 mmol) in DMF (5 mL) was added potassium carbonate (552 mg, 3.99 mmol) at ambient temperature, and the resulting mixture was warmed at 50 °C for 3 h. The mixture was poured into water and extracted with AcOEt twice. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was purified by flash column chromatography ($CHCl_3$) followed by crystallization from isopropyl ether to give **9d** (281 mg, 87.8%) as a pale-yellow solid: mp 132−134 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.34 (3H, t, J = 7.5 Hz), 2.19 (2H, quint, J = 7.5 Hz), 2.63 (2H, t, J = 7.5 Hz), 3.88 (2H, t, J = 7.5 Hz), 4.26 (2H, q, J = 7.5 Hz), 6.38 (1H, d, J = 15 Hz), 7.53 (2H, d, J = 8 Hz), 7.64 (1H, d, J = 15 Hz), 7.68 (2H, d, J = 8 Hz). Anal. ($C_{15}H_{17}NO_3$) C, H, N.

(*E*)-4-(Methanesulfonamido)cinnamic Acid (10f). To a solution of **8** (500 mg, 2.62 mmol) and triethylamine (530 mg, 5.24 mmol) in dry CH_2Cl_2 (5 mL) was added methanesulfonyl chloride (450 mg, 3.93 mmol) in an ice−water bath under nitrogen. The reaction mixture was stirred at the same temperature for 10 min and stirred at ambient temperature for 1 h. The reaction mixture was washed with saturated aqueous sodium bicarbonate, water, and brine, dried, and evaporated in vacuo. The residue was triturated with isopropyl ether to give a mixture of **9f**,**g**. This mixture was hydrolyzed following a procedure similar to method E to afford **10f** (416 mg, 65.9%) as colorless crystals after crystallization from AcOEt: mp 247−249 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 3.05 (3H, s), 6.44 (1H, d, J = 15 Hz), 7.21 (2H, d, J = 8 Hz),

7.53 (1H, d, J = 15 Hz), 7.66 (2H, d, J = 8 Hz). Anal. ($C_{10}H_{11}NO_4S$) C, H, N.

(*E*)-3-[6-(Acetylamino)pyridin-3-yl]acrylic Acid (10h). To a mixture of **11**[22] (2.15 g, 10.0 mmol) and tri-*n*-butylamine (4.08 g, 22.0 mmol) in dry xylene (10.8 mL) were added palladium(II) acetate (2.2 mg, 0.01 mmol) and triphenylphosphine (26 mg, 0.1 mmol) at ambient temperature under nitrogen. Acrylic acid (865 mg, 12.0 mmol) was added dropwise over 15 min to the refluxed mixture, and the reaction mixture was heated under reflux for 5 h. Upon cooling, H_2O (20 mL) was added therein, and the mixture was adjusted to pH 4 with 6 N HCl. After 2 h of stirring, the precipitate was collected by vacuum filtration, washed with water and isopropyl ether, and dried. The solid was crystallized from AcOEt to give **10h** (1.55 g, 75.2%) as colorless crystals: mp 291−292 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.10 (3H, s), 6.55 (1H, d, J = 16 Hz), 7.58 (1H, d, J = 16 Hz), 8.07−8.21 (2H, m), 8.59 (1H, br s). Anal. ($C_{10}H_{10}N_2O_3$) C, H, N.

Methyl (*E*)-3-(6-Aminopyridin-3-yl)acrylate (9i). To dry MeOH (5 mL) was added thionyl chloride (0.41 mL, 5.54 mmol) dropwise over 5 min in a dry ice−acetone bath under nitrogen. **10i** (700 mg, 4.26 mmol) was added therein, and the mixture was refluxed for 2 h. After cooling, the reaction mixture was concentrated in vacuo. To the residue was added water; the mixture was adjusted to pH 8 with saturated aqueous sodium bicarbonate and extracted with CH_2Cl_2. The organic layer was dried and evaporated in vacuo. The residue was crystallized from isopropyl ether to give **9i** (725 mg, 95.4%) as colorless crystals: mp 173−175 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.67 (3H, s), 6.32 (1H, d, J = 16 Hz), 6.45 (1H, d, J = 8 Hz), 6.57 (2H, s), 7.51 (1H, d, J = 16 Hz), 7.79 (1H, dd, J = 8, 2 Hz), 8.15 (1H, d, J = 2 Hz); MS (FAB) m/z 179 (M + 1). Anal. ($C_9H_{10}N_2O_2$) C, H, N.

Methyl (*E*)-3-[6-(2-Oxopyrrolidin-1-yl)pyridin-3-yl]acrylate (9k). To a solution of **9j** (270 mg, 0.825 mmol) in dry DMF (3 mL) was added 60% sodium hydride in oil (35 mg, 0.866 mmol) portionwise in an ice−water bath under nitrogen. The mixture was stirred at the same temperature for 30 min and then stirred at ambient temperature for 1 h. The mixture was poured into water and extracted with AcOEt twice. The extracts were washed with water twice and brine, dried, and evaporated in vacuo. The residue was crystallized from isopropyl ether to give **9k** (190 mg, 93.6%) as colorless crystals: mp 153−156 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.16 (2H, quint, J = 7.5 Hz), 2.69 (2H, t, J = 7.5 Hz), 3.81 (3H, s), 4.11 (2H, t, J = 7.5 Hz), 6.43 (1H, d, J = 16 Hz), 7.65 (1H, d, J = 16 Hz), 7.87 (1H, dd, J = 8, 2 Hz), 8.44−8.50 (2H, m); MS (FAB) m/z 247 (M + 1). Anal. ($C_{13}H_{14}N_2O_3$) C, H, N.

Method A. 3-Bromo-8-[[2,6-dichloro-3-[*N*-methyl-*N*-[(*N*′-phenylureido)acetyl]amino]benzyl]oxy]-2-methylimidazo[1,2-*a*]pyridine (15a). To a solution of **12**[20] (100 mg, 0.212 mmol) in dry CH_2Cl_2 (2 mL) was added phenyl isocyanate (30 mg, 0.254 mmol) at ambient temperature under nitrogen. The reaction mixture was stirred for 1 h and evaporated in vacuo. The residue was purified by flash silica gel column chromatography (CH_2Cl_2−MeOH, 50:1) followed by crystallization from AcOEt to give **15a** (74 mg, 60.3%) as colorless crystals: mp 144−147 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.42 (3H, s), 3.22 (3H, s), 3.70 (1H, dd, J = 17, 4 Hz), 3.86 (1H, dd, J = 17, 4 Hz), 5.49 (2H, s), 5.79 (1H, br t, J = 5 Hz), 6.71 (1H, d, J = 7 Hz), 6.86 (1H, t, J = 7 Hz), 7.02 (1H, m), 7.19−7.30 (5H, m), 7.32 (1H, d, J = 9 Hz), 7.42 (1H, d, J = 9 Hz), 7.78 (1H, d, J = 7 Hz). Anal. ($C_{25}H_{22}BrCl_2N_5O_3$) C, H, N.

Compounds **9e** and **15b**−**j**,**m**,**o** were prepared following a procedure similar to method A.

8-[[3-[*N*-[*N*′-(3-Aminophenyl)ureidoacetyl]-*N*-methylamino]-2,6-dichlorobenzyl]oxy]-3-bromo-2-methylimidazo[1,2-*a*]pyridine (15k). To a suspension of **16j** (800 mg, 1.26 mmol) in EtOH (8 mL) was added tin(II) chloride (954 mg, 5.03 mmol) at ambient temperature. The reaction mixture was refluxed for 2 h. After cooling, the mixture was adjusted to pH 10 with 1 N NaOH solution. To this mixture was added CH_2Cl_2 (10 mL), and the precipitate was removed by filtration with Celite. The filtrate was extracted with CH_2Cl_2 twice, and the organic layer was washed with saturated sodium bicarbonate, water, and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel chromatography (CH_2Cl_2−MeOH, 50:1) to give **15k** (539 mg, 70.6%) as a colorless amorphous solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.42 (3H, s), 3.22 (3H, s), 3.56−3.75 (3H, m), 3.82 (1H, dd, J = 18, 5 Hz), 5.48 (2H, s), 5.92 (1H, br t, J = 4 Hz), 6.88 (1H, dd, J = 8, 1 Hz), 6.53 (1H, br d, J = 7 Hz), 6.72 (1H, d, J = 7 Hz), 6.79−6.91 (2H, m), 7.01 (1H, t, J = 8 Hz), 7.09 (1H, br s), 7.33 (1H, d, J = 9 Hz), 7.45 (1H, d, J = 9 Hz), 7.78 (1H, d, J = 7 Hz); MS (FAB) m/z 607 (M + 1). Anal. ($C_{25}H_{23}BrCl_2N_6O_3$) C, H, N.

Method B. 3-Bromo-8-[[3-[*N*-(*E*)-cinnamamidoacetyl-*N*-methylamino]-2,6-dichlorobenzyl]oxy]-2-methylimidazo[1,2-*a*]pyridine (17a). To a solution of **12** (160 mg, 0.339 mmol) and triethylamine (51 mg, 0.509 mmol) in dry CH_2Cl_2 (1.6 mL) was added (*E*)-cinnamoyl chloride (62 mg, 0.373 mmol) in an ice−water bath under nitrogen. The reaction mixture was stirred at the same temperature for 30 min and stirred at ambient temperature for 1 h. The reaction mixture was washed with water, saturated aqueous sodium bicarbonate, and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel column chromatography (CH_2Cl_2−MeOH, 50:1) to give **17a** (127 mg, 60.7%) as a colorless amorphous solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.43 (3H, s), 3.29 (3H, s), 3.69 (1H, dd, J = 17, 5 Hz), 3.92 (1H, dd, J = 17, 5 Hz), 5.50 (2H, s), 6.49 (1H, d, J = 15 Hz), 6.62 (1H, br s), 6.72 (1H, d, J = 7 Hz), 6.86 (1H, t, J = 7 Hz), 7.30−7.64 (8H, m), 7.78 (1H, d, J = 7 Hz). Anal. ($C_{27}H_{23}BrCl_2N_4O_3$) C, H, N.

Compounds **9j** and **13b**−**d**,**f** were prepared following a procedure similar to method B.

Method C. 3-Bromo-8-[[2,6-dichloro-3-[*N*-[(*E*)-4-(*N*,*N*-dimethylcarbamoyl)cinnamamidoacetyl]-*N*-methylamino]benzyl]oxy]-2-methylimidazo[1,2-*a*]pyridine (17c). To a solution of **12** (350 mg, 0.741 mmol), **6b** (179 mg, 0.815 mmol), and 1-hydroxybenzotriazole (HOBt; 150 mg, 1.11 mmol) in dry DMF (3.5 mL) was added 1-ethoxy-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (WSCD·HCl; 164 mg, 0.852 mmol) portionwise at ambient temperature under nitrogen. After 2 h of stirring, this mixture was partitioned with AcOEt and saturated aqueous sodium bicarbonate solution. The organic layer was washed with water three times and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel column chromatography (AcOEt−MeOH, 20:1) followed by trituration with isopropyl ether to give **17c** (480 mg, 96.2%) as a colorless amorphous solid, which was crystallized from MeOH to afford colorless crystals (465 mg, 93.2%): mp 142−144 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.45 (3H, s), 2.99 (3H, br s), 3.10 (3H, br s), 3.29 (3H, s), 3.69 (1H, dd, J = 17, 4 Hz), 3.91 (1H, dd, J = 17, 5 Hz), 5.47 (1H, d, J = 10 Hz), 5.52 (1H, d, J = 10 Hz), 6.50 (1H, d, J = 15 Hz), 6.65 (1H, br t, J = 4 Hz), 6.71 (1H, d, J = 7 Hz), 6.86 (1H, t, J = 7 Hz), 7.29−7.62 (7H, m), 7.78 (1H, d, J = 7 Hz); MS (FAB) m/z 674 (M + 1); IR (KBr) 3491, 3275, 3054, 2931, 1675, 1625, 1549, 1513. Anal. ($C_{30}H_{28}BrCl_2N_5O_4$·$2H_2O$) C, H, N.

Compounds **9c**, **13e**, **15l**,**q**, and **17b**,**d**−**i**,**m** were prepared following a procedure similar to method C.

Method D. 3-Bromo-8-[[2,6-dichloro-3-[*N*-[(*E*)-4-(*N*,*N*-dimethylcarbamoyl)cinnamamidoacetyl]-*N*-methylamino]benzyl]oxy]-2-methylimidazo[1,2-*a*]pyridine Hydrochloride (18c). To a suspension of **17c** (400 mg, 0.594 mmol) in MeOH (4 mL) was added 10% hydrogen chloride in MeOH (0.87 mL) at ambient temperature. After 10 min of stirring, the solution was evaporated in vacuo. The residue was crystallized from AcOEt, and the product was recrystallized from MeCN to give **18c** (422 mg, 79.5%) as colorless crystals: mp 143−146 °C; ^{1}H NMR (300 MHz, $CDCl_3$−CD_3OD) δ 2.60 (3H, s), 3.02 (3H, br s), 3.12 (3H, br s), 3.30 (3H, s), 3.81 (2H, s), 5.66 (1H, d, J = 10 Hz), 5.77 (1H, d, J = 10 Hz), 6.68 (1H, d, J = 15 Hz), 7.37−7.70 (9H, m), 8.21 (1H, dd, J = 5, 2 Hz); MS (FAB) m/z 674 (M + 1); IR (KBr) 3411, 3246, 3051, 2930, 1673, 1657, 1624, 1566, 1523. Anal. ($C_{30}H_{28}BrCl_2N_5O_4$·HCl·$2H_2O$) C, H, N.

Compounds **14c**, **16a**,**c**,**e**−**j**,**m**,**p**,**q**, and **18a**−**h**,**k**−**m** were prepared following a procedure similar to method D.

Method E. 3-Bromo-8-[[3-[*N*-[(*E*)-3-(6-carboxypyridin-3-yl)acryloylglycyl]-*N*-methylamino]-2,6-dichlorobenzyl]-oxy]-2-methylimidazo[1,2-*a*]pyridine (17j). A solution of **17i** (522 mg, 0.773 mmol) in EtOH (4.2 mL) containing 1 N NaOH (0.85 mL) was heated at 60 °C for 1 h. Upon cooling, the reaction mixture was adjusted to pH 5 with 1 N HCl and diluted with water. The solid that precipitated was collected by vacuum filtration, washed with water, and dried. The solid was crystallized from MeCN to give **17i** (403 mg, 80.6%) as colorless crystals: mp 226−228 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 2.30 (3H, s), 3.16 (3H, s), 3.45−3.62 (1H, overlapped with H_2O), 3.82 (1H, dd, J = 18, 5 Hz), 5.49 (2H, s), 6.93−7.12 (3H, m), 7.51 (1H, d, J = 16 Hz), 7.79 (1H, d, J = 9 Hz), 7.85 (1H, d, J = 9 Hz), 7.93 (1H, dd, J = 5, 3 Hz), 8.01−8.20 (2H, m), 8.44 (1H, br t, J = 5 Hz), 8.89 (1H, br s); MS (FAB) m/z 648 (M + 1). Anal. ($C_{27}H_{22}BrCl_2N_5O_5$) C, H, N.

Compounds **10b**,**d**,**e**,**i**,**k** and **15n** were prepared following a procedure similar to method E.

Method F. 3-Bromo-8-[[2,6-dichloro-3-[*N*-methyl-*N*-[(*E*)-3-[6-(*N*-methylcarbamoyl)pyridin-3-yl]acryloylglycyl]amino]benzyl]oxy]-2-methylimidazo[1,2-*a*]pyridine (17k). To a solution of **17j** (100 mg, 0.154 mmol) in dry DMF (1 mL) were added methylamine hydrochloride (13 mg, 0.185 mmol), WSCD (34 mg, 0.216 mmol), and HOBt (33 mg, 0.247 mmol) at ambient temperature. After 2 h of stirring, this mixture was partitioned with AcOEt and saturated aqueous sodium bicarbonate solution. The organic layer was washed with water three times and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel column chromatography (CH_2Cl_2−MeOH, 20:1) to give **17k** (77 mg, 75.7%) as a colorless amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.45 (3H, s), 3.05 (3H, d, J = 5 Hz), 3.29 (3H, s), 3.70 (1H, dd, J = 18, 4 Hz), 3.92 (1H, dd, J = 18, 5 Hz), 5.49 (1H, d, J = 10 Hz), 5.52 (1H, d, J = 10 Hz), 6.61 (1H, d, J = 16 Hz), 6.70−6.79 (2H, m), 6.88 (1H, t, J = 7.5 Hz), 7.34 (1H, d, J = 7.5 Hz), 7.50 (1H, d, J = 7.5 Hz), 7.61 (1H, d, J = 16 Hz), 7.78 (1H, d, J = 7.5 Hz), 7.92−8.02 (2H, m), 8.02 (1H, d, J = 7.5 Hz), 8.62 (1H, br s); MS (FAB) m/z 661 (M + 1). Anal. ($C_{28}H_{25}BrCl_2N_6O_4$) C, H, N.

Compounds **5a**,**b**, **15p**, and **17l** were prepared following a procedure similar to method F.

Biological Methods. Receptor Binding: 1. Guinea Pig Ileum. The specific binding of [^{3}H]BK (a high-affinity B_2 ligand) was assayed according to the method previously described[26] 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-cold buffer (50 mM sodium (trimethylamino)ethanesulfonate (TES) and 1 mM 1,10-phenanthroline, pH 6.8) with a Polytron homogenizer. The homogenate was centrifuged to remove cellular debris (1000g, 20 min, 4 °C), and the supernatant was centrifuged (100000g, 60 min, 4 °C). The pellet was then resuspended in ice-cold binding buffer I (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. All tested compounds (2−3 mg) were dissolved in DMSO (about 0.3 mL) and then diluted with DMSO and the assay buffer (final concentration of DMSO was below 3%). 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-cold 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 in duplicate.

2. Human A-431 Cells. Human A-431 cells, human epidermoid carcinoma (obtained from the American Type Culture Collection), were maintained in Dulbecco's modified Eagle's minimum essential medium (DMEM) 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 1.5×10^5 cells/well and cultured for 1 day. The cells were washed twice with phosphate-buffered saline containing 0.1% BSA and incubated with 1.2 nM [^{3}H]BK and test compounds for 3 h at 4 °C in 0.3 mL of binding buffer II (120 mM *N*-methyl-D-glucamine, 2.68 mM KCl, 9.79 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 0.544 mM $CaCl_2$, 0.295 mM $MgCl_2$, 3.08 mM NaN_3, 2 mM bacitracin, 1 mM dithiothreitol, 1 μM captopril, and 0.1% BSA, pH 7.4). All tested compounds (2−3 mg) were dissolved in DMSO (about 0.3 mL) and then diluted with DMSO and the assay buffer (final concentration of DMSO was below 3%). All experiments were carried out three times in duplicate. At the end of the incubation, the buffer was aspirated, and the cells were washed three times with ice-cooled phosphate-buffered saline containing 0.1% BSA. The cells were solubilized with 1% sodium dodecyl sulfate (SDS) and 0.1 N NaOH, and radioactivity was determined by a liquid scintillation counter. The specific binding was calculated by subtracting the nonspecific binding, determined in the presence of 1 μM unlabeled BK, from the total binding.

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), and the trachea, jugular vein, and esophagus 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 Rössler method[27] as the peak increase of pulmonary insufflation pressure (PIP).[28] Each dose of the compound suspended in 0.5% methylcellulose solution (5 mL/kg) or vehicle was administered through the esophageal cannula after the first BK-induced bronchoconstriction. After 30 min, BK was administered again and 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 = ($\Delta PIP_{after\ drug}/\Delta PIP_{before\ drug}$) × 100. Percent response obtained from the vehicle-administered animals was regarded as the control. Three or four animals were used in each dose. The potency of the drug was expressed as percent inhibition which was calculated from the values of percent responses of drug-treated and control groups as follows: % inhibition = (1 − % $response_{drug}$/% $response_{mean\ value\ of\ vehicle}$) × 100.

Statistical Analysis. Statistical significance was analyzed with the results of percent inhibition between groups by Student's t-test. IC_{50} or ED_{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 of **5a**,**b**, **6a**,**b**, **7b**, **9c**,**e**,**j**, **10b**,**d**,**e**,**i**,**k**, **13b**−**f**, **14c**, **15b**−**j**,**l**−**q**, **16a**,**c**,**e**−**j**,**m**,**p**,**q**, **17b**,**d**−**i**,**m**, and **18a**−**h**,**k**−**m** (19 pages). Ordering information is given on any current masthead page.

References

(1) Regoli, D.; Barabé, J. Pharmacology of Bradykinin and Related Kinins. *Pharmacol. Rev.* **1980**, *32*, 1−46.

(2) Marceau, F.; Lussier, A.; Regoli, D.; Giroud, J. P. Pharmacology of Kinins: Their Relevance to Tissue Injury and Inflammation. *Gen. Pharmacol.* **1983**, *14*, 209−229.

(3) Proud, D.; Kaplan, A. P. Kinin Formation: Mechanisms and Role in Inflammatory Disorders. *Annu. Rev. Immunol.* **1988**, *6*, 49−83.

(4) Dray, A.; Perkins, M. Bradykinin and Inflammatory Pain. *Trends Neurosci.* **1993**, *16*, 99−104.

(5) Greaves, M. W. Inflammation and Mediators. *Br. J. Dermatol.* **1988**, *119*, 419−426.

(6) Bhoola, K. D.; Figueroa, C. D.; Worthy, K. Bioregulation of Kinins: Kallikreins, Kininogens and Kininases. *Pharmacol. Rev.* **1992**, *44*, 1−80.

(7) Farmer, S. G.; Burch, R. M. The Pharmacology of Bradykinin Receptors. In *Bradykinin Antagonists: Basic and Clinical Research*; Burch, R. M., Ed.; Marcel Dekker: New York, 1991; pp 1−31.

(8) Burch, R. M.; Farmer, S. G.; Steranka, L. R. Bradykinin Receptor Antagonists. *Med. Res. Rev.* **1990**, *10*, 237−269.

(9) McEachern, A. E.; Shelton, E. R.; Bhakta, S.; Obernolte, R.; Bach, C.; Zuppan, P.; Fujisaki, J.; Aldrich, R. W.; Jarnagin, K. Expression Cloning of a Rat B_2 Bradykinin Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 7724−7728.

(10) Hess, J. F.; Borkowski, J. A.; Young, G. S.; Strader, C. D.; Ransom, R. W. Cloning and Pharmacological Characterization of a Human Bradykinin (BK-2) Receptor. *Biochem. Biophys. Res. Commun.* **1992**, *184*, 260−268.

(11) Menke, J. G.; Borkowski, J. A.; Bierilo, K. K.; MacNeil, T.; Derrick, A. W.; Schneck, K. A.; Ransom, R. W.; Strader, C. D.; Linemeyer, D. L.; Hess, J. F. Expression Cloning of a Human B_1 Bradykinin Receptor. *J. Biol. Chem.* **1994**, *269*, 21583−21586.

(12) Hock, F. J.; Wirth, K.; Albus, U.; Linz, W.; Gerhards, H. J.; Wiemer, G.; Henke, S.; Breipohl, G.; König, W.; Knolle, J.; Schölkens, B. A. Hoe 140 A New Potent and Long Acting Bradykinin-Antagonist: In Vitro Studies. *Br. J. Pharmacol.* **1991**, *102*, 769−773.

(13) Wirth, K.; Hock, F. J.; Albus, U.; Linz, W.; Alpermann, H. G.; Anagnostopoulos, H.; Henke, St.; Breipohl, G.; König, W.; Knolle, J.; Schölkens, B. A. Hoe 140 A New Potent and Long Acting Bradykinin-Antagonist: In Vivo Studies. *Br. J. Pharmacol.* **1991**, *102*, 774−777.

(14) Cheronis, J. C.; Whalley, E. T.; Nguyen, K. T.; Eubanks, S. R.; Allen, L. G.; Duggan, M. J.; Loy, S. D.; Bonham, K. A.; Blodgett, J. K. A New Class of Bradykinin Antagonists: Synthesis and In Vitro Activity of Bissuccinimidoalkane Peptide Dimers. *J. Med. Chem.* **1992**, *35*, 1563−1572.

(15) Kyle, D. J.; Martin, J. A.; Burch, R. M.; Carter, J. P.; Lu, S.; Meeker, S.; Prosser, J. C.; Sullivan, J. P.; Togo, J.; Noronha-Blob, L.; Sinsko, J. A.; Walters, R. F.; Whaley, L. W.; Hiner, R. N. Probing The Bradykinin Receptor: Mapping The Geometric Topography Using Ethers of Hydroxyproline in Novel Peptides. *J. Med. Chem.* **1991**, *34*, 2649−2653.

(16) Kyle, D. J. Structure-Based Drug Design: Progress Toward The Discovery of The Elusive Bradykinin Receptor Antagonists. *Curr. Pharm. Des.* **1995**, *1*, 233−254.

(17) Calixto, J. B.; Yunes, R. A.; Rae, G. A.; Medeiros, Y. S. Nonpeptide Bradykinin Antagonists. In *Bradykinin Antagonists, Basic and Clinical Research*; Burch, R. M., Ed.; Marcel Dekker: New York, 1991; pp 97−129.

(18) Salvino, J. M.; Seoane, P. R.; Douty, B. D.; Awad, M. M. A.; Dolle, R. E.; Houck, W. T.; Faunce, D. M.; Sawutz, D. G. Design of Potent Non-Peptide Competitive Antagonists of the Human Bradykinin B_2 Receptor. *J. Med. Chem.* **1993**, *36*, 2583−2584.

(19) Hoyer, D.; Awad, M. M. A.; Salvino, J. M.; Seoane, P. R.; Dolle, R. E.; Houck, W. T.; Sawutz, D. G. ACE Inhibitors as a Template for the Design of Bradykinin B_2 Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 367−370.

(20) Abe, Y.; Kayakiri, H.; Satoh, S.; Inoue, T.; Sawada, Y.; Imai, K.; Inamura, N.; Asano, M.; Hatori, C.; Katayama, A.; Oku, T.; Tanaka, H. A Novel Class of Orally Active Non-Peptide Bradykinin B_2 Receptor Antagonists. 1. Construction of the Basic Framework. *J. Med. Chem.* **1998**, *41*, 564−578.

(21) (a) Inamura, N.; Asano, M.; Hatori, C.; Sawai, H.; Hirosumi, J.; Fujiwara, T.; Kayakiri, H.; Satoh, S.; Abe, Y.; Inoue, T.; Sawada, Y.; Oku, T.; Nakahara, K. Pharmacological Characterization of a Novel, Orally active, Nonpeptide Bradykinin B_2 Receptor Antagonist, FR167344. *Eur. J. Pharmacol.* **1997**, *333*, 79−86. (b) Aramori, I.; Zenkoh, J.; Morikawa, N.; O'Donnell, N.; Asano, M.; Nakamura, K.; Iwami, M.; Kojo, H.; Notsu, Y. Novel Subtype-Selective Nonpeptide Bradykinin Receptor Antagonists FR167344 and FR173657. *Mol. Pharmacol.* **1997**, *51*, 171−176. (c) Asano, M.; Hatori, C.; Inamura, N.; Sawai, H.; Hirosumi, J.; Fujiwara, T.; Nakahara, K. Effects of a Nonpeptide Bradykinin B_2 Receptor Antagonist, FR167344, on Different in vivo Animal Models of Inflammation. *Br. J. Pharmacol.* **1997**, *122*, 1436−1440.

(22) Igarashi, Y.; Shimoyamada, M.; Takashima, H.; Suzuki, T.; Watanabe, S. Novel Bromination Methods of 2-Acetamidopyridines. *Nihonkagakukaishi* **1992**, *5*, 586−589.

(23) Later we confirmed that the binding affinities of our B_2 antagonists for the B_2 receptors in A-431 cells were well-correlated to those for cloned human B_2 receptors. The results will be reported in the following paper (see ref 24).

(24) For part 3 of this series, see: Abe, Y.; Kayakiri, H.; Satoh, S.; Inoue, T.; Sawada, Y.; Inamura, N.; Asano, M.; Aramori, I.; Hatori, C.; Sawai, H.; Oku, T.; Tanaka, H. A Novel Class of Orally Active Non-Peptide Bradykinin B_2 Receptor Antagonists. 3. Discovering Bioisosteres of the Imidazo[1,2-*a*]pyridine Moiety. *J. Med. Chem.* **1998**, *41*, 4062−4079.

(25) (a) Matsumoto, I.; Yoshizawa, J. Japan Kokai 48-54072, 1973; *Chem. Abstr.* **1973**, *79*, 105071h. (b) Matsumoto, I.; Tomimoto, K. Japan Kokai 49-61171, 1974.

(26) Manning, D. C.; Vavrek, R.; Stewart, J. M.; Synder, S. H. Two Bradykinin Binding Sites with Picomolar Affinities. *J. Pharmacol. Exp. Ther.* **1986**, *237*, 504−512.

(27) Konzett, H.; Rössler, R. Versuchsanordnung zu Untersuchungen an der Bronchialmuskulatur. *Naunyn-Schmiedeberg's Arch. Exp. Path. Pharmak.* **1940**, *195*, 71−74.

(28) Asano, M.; Inamura, N.; Nakahara, K.; Nagayoshi, A.; Isono, T.; Hamada, K.; Oku, T.; Notsu, Y.; Kohsaka, M.; Ono, T. A 5-Lipoxygenase Inhibitor, FR110302, Suppresses Airway Hyperresponsiveness and Lung Eosinophilia Induced by Sephadex Particles in Rats. *Agents Actions* **1992**, *36*, 215−221.

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