A Novel Class of Orally Active Non-Peptide Bradykinin B_2 Receptor Antagonists. 3. Discovering Bioisosteres of the Imidazo[1,2-*a*]pyridine Moiety

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Received May 13, 1998

Recently we reported on overcoming the species difference of our first orally active non-peptide bradykinin (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, leading to identification of the first clinical candidate **4a** (FR167344). With this potent new lead compound in hand, we then investigated further refinement of the basic framework by replacement of the imidazo[1,2-*a*]pyridine moiety and discovered several bioisosteric heterocycles. Extensive optimization of these new heteroaromatic derivatives revealed the detailed structure−activity relationships (SAR) around the imidazo[1,2-*a*]pyridine ring and the 2,6-dichlorobenzyl moiety, leading to the discovery of our second clinical candidate **87b** (FR173657) which inhibited the specific binding of [^{3}H]BK to recombinant human B_2 receptors expressed in Chinese hamster ovary (CHO) cells and guinea pig ileum membrane preparations expressing B_2 receptors with IC_{50}'s of 1.4 and 0.46 nM, respectively. This compound also displayed excellent in vivo functional antagonistic activity against BK-induced bronchoconstriction in guinea pigs with an ED_{50} value of 0.075 mg/kg by oral administration. Further modifications of the terminal substituents on the pyridine moiety led to a novel pharmacophore and resulted in the identification of **99** (FR184280), whose IC_{50} value for human B_2 receptors (0.51 nM) was comparable to that of the second-generation peptide B_2 antagonist Icatibant.

Introduction

Bradykinin (BK), an endogenous nonapeptide, has been implicated in a variety of pathophysiological responses, including pain, inflammation, asthma, rhinitis, and hypotension.[1−8] Classification of BK receptors into B_1 and B_2 receptors is widely accepted, and they 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]

Since the discovery of the first BK B_2 receptor antagonist by Vavrek and Stewart in 1985,[12] a number of peptide B_2 antagonists have been developed,[13−17] including the clinically evaluated second-generation antagonists Icatibant (Hoe140)[13,14] and Bradycor (CP0127).[15] Despite their highly potent B_2 antagonistic activity, therapeutic use is still limited because of their peptidic nature. On the other hand, few non-peptide antagonists have been disclosed.[18−20] However, until our work[21] there had been no reports of potent orally active non-peptide B_2 antagonists.

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 BK B_2 receptor antagonists **1**−**3** (Chart 1).[21] Although their affinities for B_2 receptors in human A-431 cells (human epidermoid carcinoma) were found to be very low, the discovery of a novel key pharmacophore enabled us to overcome the species difference and to identify the first clinical candidate **4a** (FR167344).[22,23] In the present study we explored replacement of the imidazo[1,2-*a*]pyridine moiety, which has been incorporated in all of our B_2 antagonists, with the aim of further refinement of the basic framework and discovered several promising bioisosteres. Extensive optimization of these new heteroaromatic derivatives led us to identify three series of potent novel B_2 antagonists, with nanomolar IC_{50}'s for the human recombinant B_2 receptors expressed in CHO cells.[22b] In particular, the quinoline series afforded the second clinical candidate, **87b** (FR173657),[22b,24] with excellent in vivo pharmacological profiles and a subnanomolar B_2 antagonist, **99** (FR184280), with a novel pharmacophore. Herein we wish to describe the structure−activity relationships (SAR) revealed on the way to the discovery of **87b** and **99**.

Chemistry

The compounds described in this study are shown in Tables 1−5, and their synthetic methods are outlined in Schemes 1−8.

Schemes 1−5 show the synthetic routes for the various heteroaromatic alcohols, which were introduced to the basic framework in place of the imidazo[1,2-*a*]-pyridin-8-ol moiety. Synthesis of 1,2-disubstituted-4-hydroxy-1*H*-benzimidazole derivatives was accomplished

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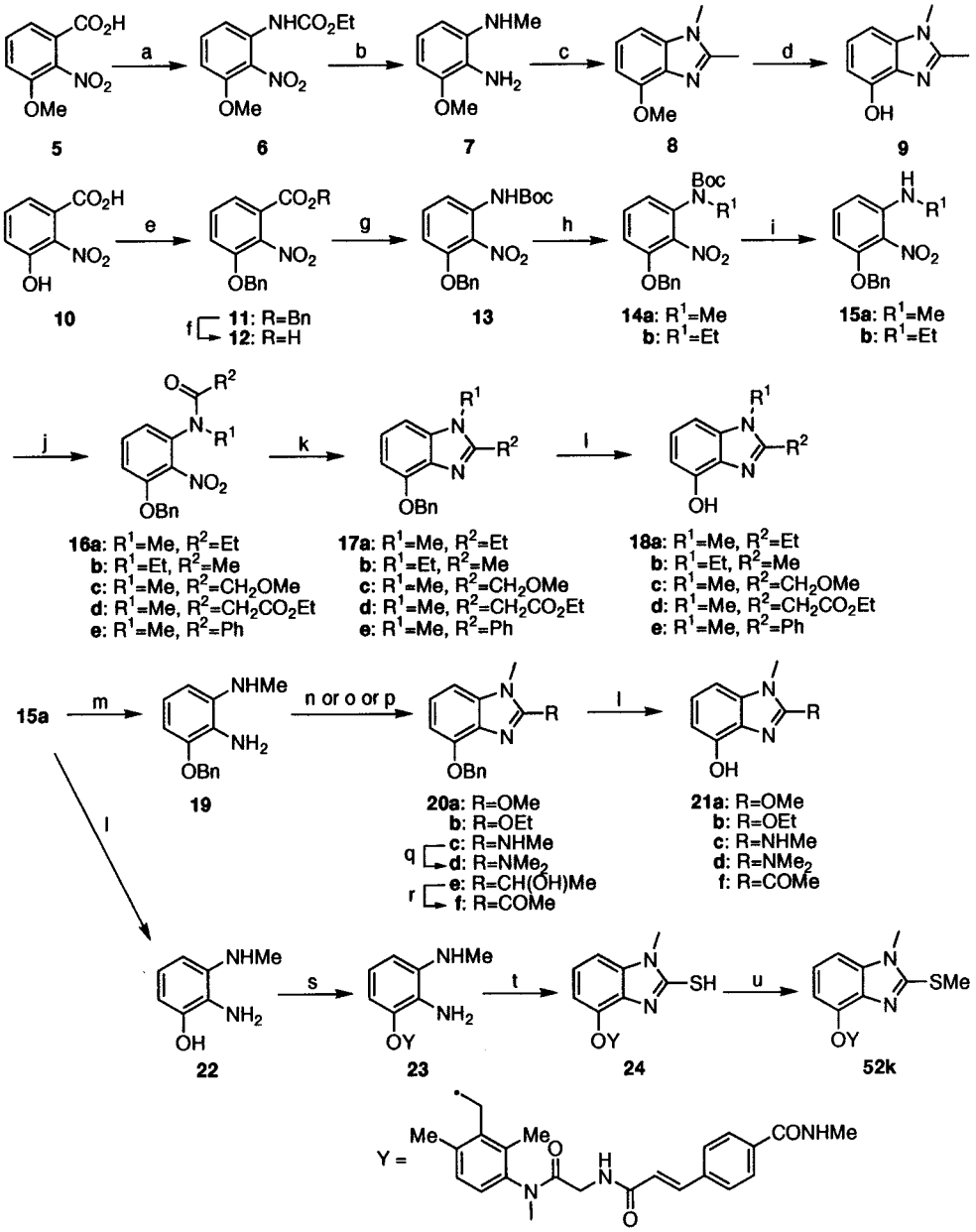
S0022-2623(98)00300-8 CCC: $15.00
Published on Web 09/19/1998

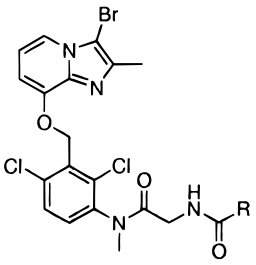
Scheme 1[a]



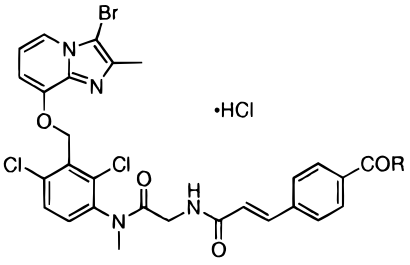
[a] (a) DPPA, Et_3N, benzene then EtOH; (b) LAH, THF; (c) AcOH, 4 N HCl; (d) BBr_3, CH_2Cl_2; (e) BnBr, K_2CO_3, DMF; (f) 1 N NaOH, EtOH; (g) DPPA, Et_3N, benzene then *t*-BuOH; (h) R^1I, NaH, DMF; (i) 4 N HCl–AcOEt; (j) R^2COCl, $PhNMe_2$; (k) Fe, AcOH, EtOH; (l) H_2, Pd–C; (m) $N_2H_4 \cdot H_2O$, $FeCl_3 \cdot 6H_2O$, C, aqueous MeOH; (n) CR_4, AcOH; (o) MeNCS, THF then MeI, MeCN; (p) $MeCH(OH)CO_2H$, 4 N HCl; (q) MeI, NaH, DMF; (r) MnO_2, CH_2Cl_2; (s) **50b**, NaH, DMF; (t) 1,1′-thiocarbonylimidazole, THF; (u) MeI, K_2CO_3, DMF.

in the following way (Scheme 1). 3-Methoxy-2-nitrobenzoic acid (**5**) was converted to the carbamate **6** by Curtius rearrangement in the presence of diphenyl phosphorazidate (DPPA), triethylamine, and EtOH. Reduction of both the ethyl carbamate and the nitro groups of **6** with lithium aluminum hydride gave the diamine **7**, which was cyclized with AcOH in 4 N hydrochloric acid followed by removal of the *O*-methyl group with boron tribromide to afford the 1,2-dimethylbenzimidazole **9**. Next, introduction of various substituents at the 2-position of the benzimidazole ring was investigated. Protection of 3-hydroxy-2-nitrobenzoic acid (**10**) with benzyl bromide in the presence of potassium carbonate, alkaline hydrolysis of the benzyl ester **11**, and subsequent Curtius rearrangement with DPPA and *t*-BuOH furnished the *N*-Boc derivative **13**. Alkylation of **13** with methyl iodide or ethyl iodide using sodium hydride as a base gave **14a**,**b**. After removal of the *N*-Boc group of **14a**,**b**, the amino group of **15a**,**b** could be coupled with various acid chlorides smoothly to afford the amides **16a**–**e**. Reductive cyclization of **16a**–**e** with iron followed by catalytic deprotection of the benzyl group provided the 4-hydroxybenzimidazoles **18a**–**e**. The diamine **19** was prepared by reduction of the nitro group of **15a** with hydrazine monohydrate, iron(II) chloride hexahydrate, and carbon in aqueous MeOH. Cyclization of **19** with appropriate orthoesters and subsequent hydrogenolysis gave the 2-alkoxybenzimidazole derivatives **21a**,**b**. Reaction of **19** with methyl isothiocyanate followed by treatment with methyl iodide in MeCN furnished the expected 2-methylamino analogue **20c**, which could be alkylated to the dimethylamine **20d** with methyl iodide and sodium hydride in DMF. The 2-amino derivatives **20c**,**d** were

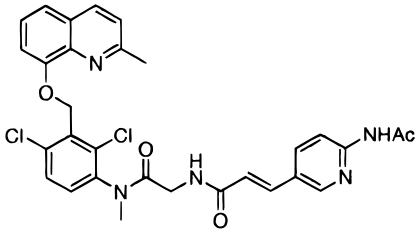
Chart 1



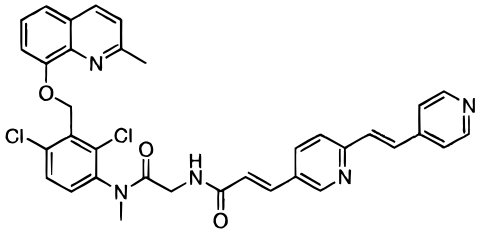
1: R=*n*-Pr
2: R=CH_2NMe_2
3: R=NHEt



4a (FR167344): R=NMe_2
4b : R=NHMe

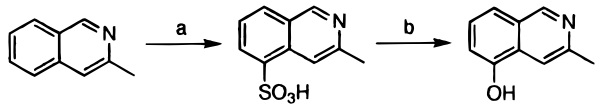


87b (FR173657)



99 (FR184280)

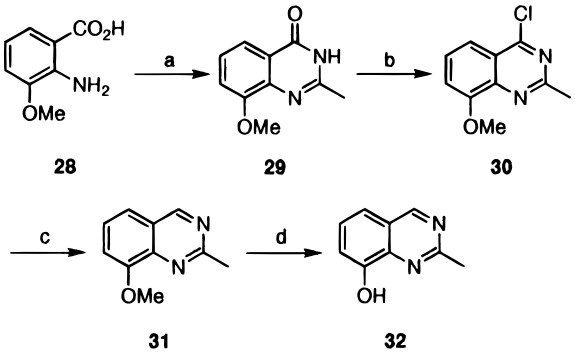
Scheme 2[a]



25 **26** **27**

[a] (a) Concentrated H_2SO_4, 50% fuming H_2SO_4; (b) KOH, NaOH, 210 °C.

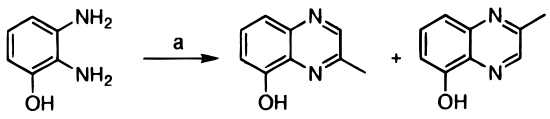
Scheme 3[a]



28 **29** **30** **31** **32**

[a] (a) MeCOCl, DMAP, Et_3N, DMF then $(NH_4)_2CO$; (b) $PhNMe_2$, $POCl_3$, 60 °C; (c) H_2, Pd−C, Et_3N, AcOEt; (d) BBr_3, CH_2Cl_2.

Scheme 4[a]

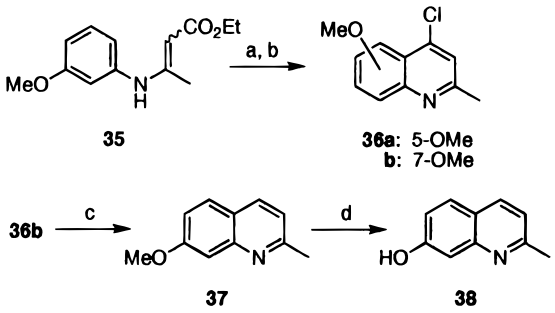


33 **34a** **34b**

[a] (a) AcCHO, 2 M AcOH, 4 M AcONa, 60 °C.

deprotected to provide **21c**,**d**. The 2-acetyl derivative **21f** was prepared by cyclization of **19** with lactic acid in 4 N HCl followed by oxidation and deprotection. Hydrogenation of **15a**, alkylation of the phenol **22** with the benzyl chloride **50b** in the presence of sodium hydride, and subsequent cyclization with 1,1′-thiocarbonylimidazole gave the 2-mercapto derivative **24**. The thiol group of **24** was methylated with methyl iodide using potassium carbonate as a base to afford the 2-methylthio derivative **52k**.

Scheme 5[a]



35 **36a**: 5-OMe **b**: 7-OMe **37** **38**

[a] (a) Dowtherm, 250 °C; (b) $PhNMe_2$, $POCl_3$, 60 °C; (c) H_2, Pd−C, Et_3N, AcOEt; (d) BBr_3, CH_2Cl_2.

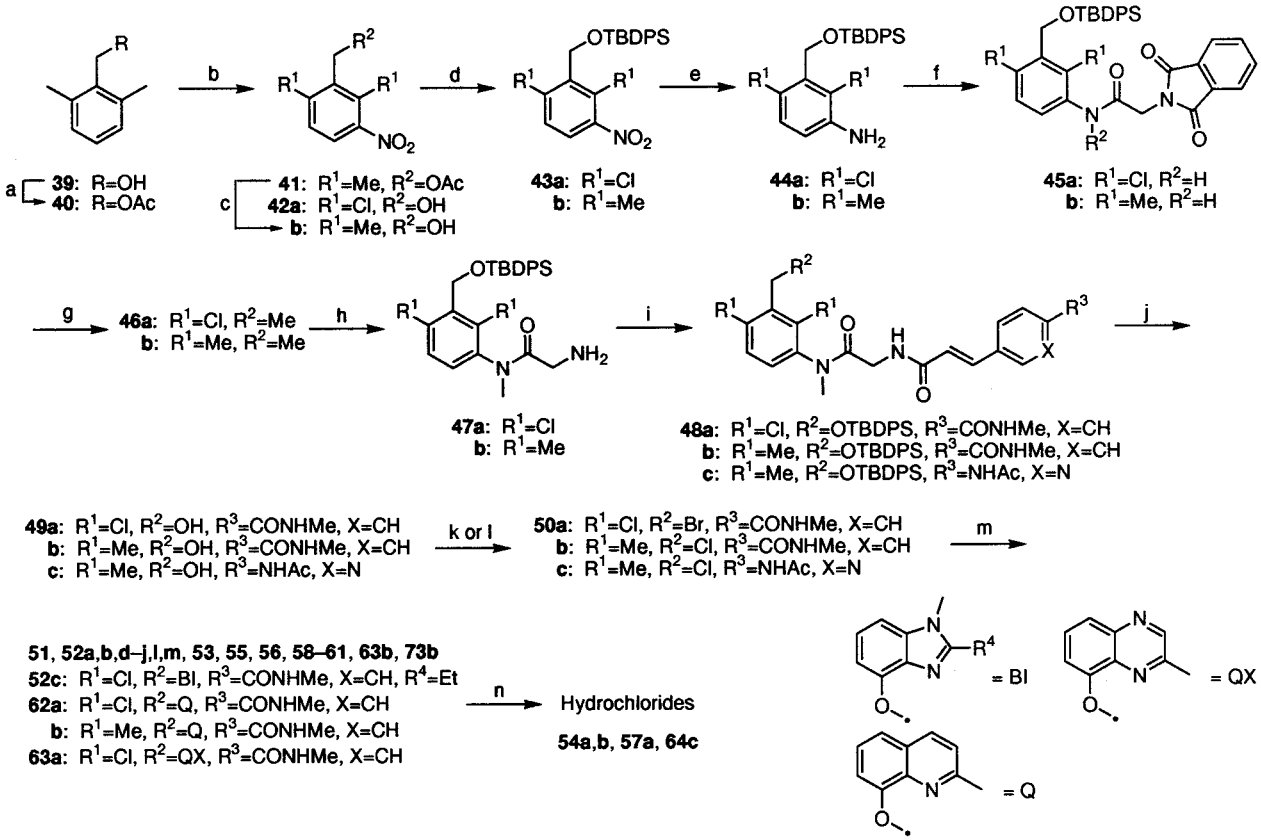
5-Hydroxy-3-methylisoquinoline (**27**) was prepared by sulfonation at the 5-position of 3-methylisoquinoline (**25**), followed by alkaline fusion (Scheme 2).[25]

Synthesis of 8-hydroxy-2-methylquinazoline (**32**) is shown in Scheme 3. Cyclization of 2-amino-3-methoxybenzoic acid (**28**) with acetyl chloride in the presence of 4-(dimethylamino)pyridine and triethylamine in DMF followed by treatment with ammonium carbonate furnished the quinazolone derivative **29**. Reaction of **29** with phosphorus oxychloride and subsequent catalytic hydrogenation gave the quinazoline **31**, which was deprotected by treatment with boron tribromide to afford **32**.

Cyclization of 2,3-diaminophenol (**33**) with pyruvic aldehyde provided a ca. 1:1 mixture of 2- and 3-methylquinoxaline derivatives **34a**,**b**, which were separated by silica gel column chromatography (Scheme 4). The structural assignments of these isomers were based on the X-ray crystallography of 2-methyl isomer **34a**.

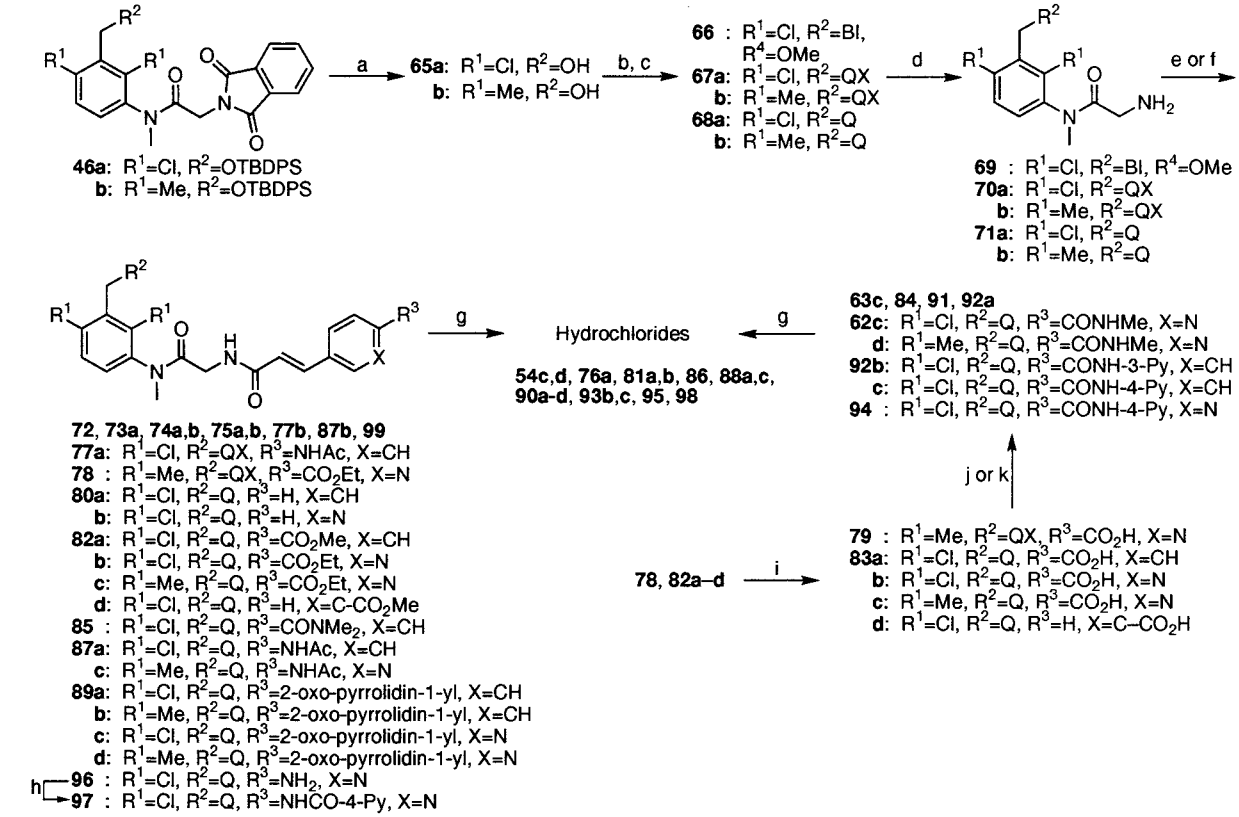
Synthesis of 7-hydroxy-2-methylquinoline (**38**) is shown in Scheme 5. The crotonate **35**, which was prepared by

Scheme 6[a]



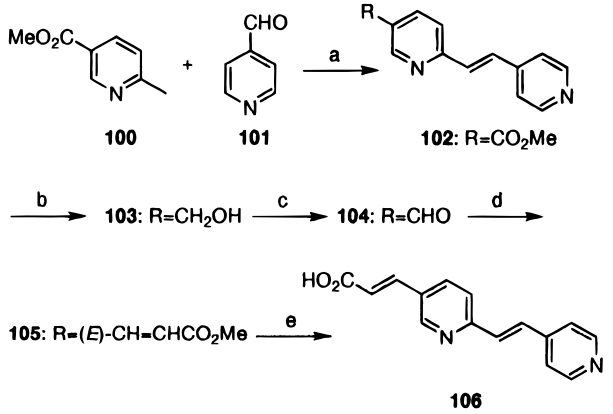
[a] (a) Ac_2O, DMAP; (b) $Cu(NO_3)_2 \cdot 3H_2O$, Ac_2O; (c) 1 N NaOH, MeOH; (d) TBDPSCl, imidazole, DMF; (e) $N_2H_4 \cdot H_2O$, $FeCl_3 \cdot 6H_2O$, C, aqueous MeOH; (f) *N*-phthaloylglycyl chloride, pyridine, DMF; (g) MeI, NaH, DMF; (h) $N_2H_4 \cdot H_2O$, EtOH; (i) (*E*)-4-(*N*-methylcarbamoyl)cinnamic acid or (*E*)-3-(6-acetamidopyridin-3-yl)acrylic acid, WSCD·HCl, HOBt, DMF; (j) *n*-Bu_4NF, THF; (k) PPh_3, CBr_4, CH_2Cl_2; (l) MsCl, Et_3N, DMF; (m) heterocyclic alcohols (BI-H, QX-H, Q-H), NaH, DMF; (n) HCl−MeOH.

Scheme 7[a]



[a] (a) *n*-Bu_4NF, THF; (b) MsCl, Et_3N, CH_2Cl_2; (c) heterocyclic alcohols (BI-H, QX-H, Q-H), NaH, DMF; (d) $N_2H_4 \cdot H_2O$, EtOH; (e) (*E*)-cinnamoyl chloride, Et_3N, CH_2Cl_2; (f) substituted acrylic acids, WSCD·HCl, HOBt, DMF; (g) HCl−MeOH; (h) 4-PyCOCl·HCl, Et_3N, CH_2Cl_2; (i) 1 N NaOH, EtOH; (j) $MeNH_2 \cdot HCl$, WSCD, HOBt, DMF; (k) 2-, 3-, 4-$PyNH_2$ or $PhNH_2$, WSCD·HCl, HOBt, DMF.

Scheme 8[a]



[a] (a) Ac_2O, AcOH; (b) LAH, THF; (c) SO_3·Py, DMSO, CH_2Cl_2; (d) $Ph_3P{=}CHCO_2Me$, THF; (e) 1 N NaOH, MeOH.

condensing *m*-anisidine with ethyl acetoacetate in benzene with a catalytic amount of AcOH, was cyclized in Dowtherm at 250 °C to give a 2:7 mixture of 5- and 7-methoxy-2-methyl-4-quinolones. The mixture was treated with phosphorus oxychloride followed by silica gel column separation to give 4-chloro-5-methoxy-2-methylquinoline (**36a**) and 4-chloro-7-methoxy-2-methylquinoline (**36b**), respectively. Catalytic hydrogenation of **36b** followed by deprotection with boron tribromide yielded the 7-quinolinol **38**.

Introduction of various heteroaromatic rings in place of the imidazo[1,2-*a*]pyridine ring onto the basic framework is shown in Scheme 6. Protection of 2,4-dichloro-3-nitrobenzyl alcohol (**42a**)[21] with the silyl group followed by reduction of the nitro group with hydrazine monohydrate, iron(III) chloride hexahydrate, and carbon gave the aniline **44a**. The *N*-phthaloylglycinamide **45a** was obtained from **44a** by coupling with *N*-phthaloylglycyl chloride in the presence of pyridine in DMF. Alkylation of **45a** with methyl iodide and sodium hydride yielded **46a**. The *N*-phthaloyl group was deprotected with hydrazine monohydrate, and the resulting amine **47a** was condensed with (*E*)-4-(*N*-methylcarbamoyl)cinnamic acid[23] to give the cinnamamide **48a**. Removal of the silyl protecting group with tetra-*n*-butylammonium fluoride and subsequent treatment with triphenylphosphine and carbon tetrabromide in CH_2Cl_2 provided the benzyl bromide **50a**. Coupling of **50a** with various heteroaromatic alcohols was performed with sodium hydride as a base to give **51**, **52a**−**e**, **53**, **55**, **56**, **58**−**61**, **62a**, and **63a**, respectively. The hydrochlorides **54a**, **57a**, and **64c** were obtained by treatment of **62a**, **63a**, and **52c** with 10% hydrogen chloride in MeOH. Acetylation of 2,6-dimethylbenzyl alcohol (**39**) and selective nitration at the 3-position with copper(II) nitrate followed by hydrolysis gave the 3-nitrobenzyl alcohol derivative **42b**. Subsequent silylation, reduction, coupling, and methylation provided the *N*-phthaloylglycinamide derivative **46b**. Treatment of **46b** with hydrazine monohydrate and coupling with (*E*)-4-(*N*-methylcarbamoyl)cinnamic acid[23] or (*E*)-3-(6-acetamidopyridin-3-yl)acrylic acid[23] gave the cinnamamides **48b**,**c**, which were deprotected with fluoride to yield the benzyl alcohols **49b**,**c**. Reaction with methanesulfonyl chloride and coupling with the substituted benzimidazolols **18c**,**d** and **21a**−**d**,**f**, quinoxalinol **34a**, and 2-methyl-8-quinolinol afforded **52f**−**j**,**l**,**m**, **62b**, **63b**, and **73b**, respectively. The quinoline derivative **62b** was converted to the hydrochloride **54b**.

Modifications of the terminal cinnamamide and pyridyl acrylamide moiety of benzimidazoles, quinoxalines, and quinolines are shown in Scheme 7. Deprotection of the silyl moiety of the *N*-phthaloylglycinamides **46a**,**b** with tetra-*n*-butylammonium fluoride and subsequent treatment with methanesulfonyl chloride followed by coupling with benzimidazolol **21a**, quinoxalinol **34a**, and 2-methyl-8-quinolinol gave **66**, **67a**,**b**, and **68a**,**b**, respectively. Removal of the *N*-phthaloyl group of **66**, **67a**,**b**, and **68a**,**b** with hydrazine monohydrate and coupling with (*E*)-cinnamoyl chloride, (*E*)-3- or 4-(substituted)cinnamic acids,[23] or (*E*)-3-[6-(substituted)pyridin-3-yl]acrylic acids[23] afforded the corresponding cinnamamides **72**, **74a**,**b**, **75a**,**b**, **80a**, **82a**,**d**, **85**, **87a**, and **89a**,**b** and the pyridyl acrylamides **73a**, **77a**,**b**, **78**, **80b**, **82b**,**c**, **87b**,**c**, **89c**,**d**, **96**, and **99**, respectively. The amine **96** was acylated with isonicotinoyl chloride to give the amide **97**. The esters **78** and **82a**−**d** were hydrolyzed and condensed with the corresponding amines to afford the amides **62c**,**d**, **63c**, **84**, **91**, **92a**−**c**, and **94**, respectively. The quinoxaline **77a** and the quinoline derivatives **62c**,**d**, **80a**,**b**, **85**, **87a**,**c**, **89a**−**d**, **92b**,**c**, **94**, and **97** were treated with 10% hydrogen chloride in MeOH to give the corresponding hydrochlorides **54c**,**d**, **76a**, **81a**,**b**, **86**, **88a**,**c**, **90a**−**d**, **93b**,**c**, **95**, and **98**, respectively.

Preparation of the acrylic acid **106**, which was coupled with **71a** to synthesize **99** in Scheme 7, is shown in Scheme 8. Condensation of methyl 6-methylnicotinate (**100**) with 4-pyridinecarboxaldehyde (**101**) gave the (*E*)-vinylenedipyridine **102**. Reduction of the ester group of **102** with lithium aluminum hydride followed by oxidation with sulfur trioxide pyridine complex in DMSO and CH_2Cl_2 afforded the aldehyde **104**. Wittig reaction of **104** and methyl (triphenylphosphoranylidene)acetate afforded the ester **105**, which was hydrolyzed to give the acid **106**. Synthesis of the other acrylic acids employed in Schemes 6 and 7 was previously described.[23]

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,[21−23,24a] and they were also evaluated for inhibition of the specific binding of [^{3}H]BK to B_2 receptors in human A-431 cells[23] or human recombinant B_2 receptors expressed in CHO cells.[22b] 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.[21,22a,23,24a]

Results and Discussion

Recently we reported 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.[21] 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 from 1 mg/kg by oral administration. However, it was revealed that our non-peptide B_2 antagonists showed only weak binding affinities in the human A-431 cell system. Intensive modifications of the terminal substituents at the glycine moiety allowed us to discover a novel key pharmacophore to overcome this species difference, namely, the (*E*)-4-(substituted)cinnamamide and (*E*)-3-[6-(substituted)pyridin-3-yl]acrylic acid moieties, leading to the discovery of the first clinical candidate **4a**.[22,23] Using **4a** as a new lead compound, we then investigated further refinement of the basic framework by replacement of the imidazo[1,2-*a*]pyridine moiety, which has been incorporated in all our B_2 antagonists, with other heteroaromatic rings. To reconfirm the B_2 binding affinity for human receptors, we introduced a new binding assay system with recombinant human B_2 receptors expressed in CHO cells.[22b] Table 1 summarizes the results, indicating that the representative imidazo[1,2-*a*]pyridine derivatives **4a**,**b** also exhibited potent activities in this system, with nanomolar IC_{50}'s, and that the binding affinities for both types of human B_2 receptors are consistent in a wide range of compounds. In the 5−6 condensed-ring systems, benzimidazole derivative **52a** retained a 7-fold weaker binding affinity to human recombinant B_2 receptors compared with imidazo[1,2-*a*]pyridine **4b**. However, benzoxazole (**51**) and benzofuan (**53**) derivatives showed a remarkable decrease in the activity. On the other hand, in the 6−6 condensed-ring systems, the quinoline ring (**54a**) afforded a 8-fold increase in affinity for human recombinant B_2 receptors with an IC_{50} value of 1.1 nM. This promising result prompted us to further investigate the quinoline and related heteroaromatic derivatives. Transposition of the nitrogen atom from the 1-position to the 3-position within the quinoline ring gave the inactive isoquinoline **55**. Introduction of another nitrogen atom at the 3-position provided the quinazoline **56** which displayed a 50-fold decreased binding activity in the human recombinant system. In contrast, incorporation of a second nitrogen atom at the 4-position led to quinoxaline derivative **57a** which retained good activity. However, the 3-methyl derivative **58** was 558-fold less potent than the 2-methyl derivative **57a**, suggesting that the 2-methyl group interacts with the hydrophobic pocket of the B_2 receptor consistent with our previously discussed hypothesis.[21] Shifting the benzyloxy moiety from the 8-position to the 7-position (**59**) resulted in complete loss of B_2 binding affinity. These results indicate that, according to the numbering of the imidazo[1,2-*a*]pyridine ring, the 1-nitrogen atom makes an essential electrostatic interaction with B_2 receptors, while the 4-nitrogen atom is unnecessary. Moreover, the 3-substituent, which increases the affinity in the 5−6 condensed ring systems, is not necessary in the 6−6 condensed-ring systems. Replacement of the condensed-ring with the monocyclic rings (**60**, **61**) resulted in a significant loss of binding activity. Thus we selected benzimidazole **52a**, quinoline **54a**, and quinoxaline **57a** as new lead compounds for further chemical modifications.

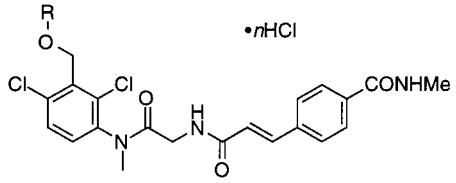
Table 2 shows the effects of modifications in the benzimidazole series on their B_2 binding activities. Enlargement of the 1-methyl group of **52a** to an ethyl group (**52b**) slightly increased the affinity for human recombinant B_2 receptors. The same enlargement at the 2-position of **52a** (**64c**) provided 3-fold potentiation. It was remarkable that replacement of the 2-methyl group of **52a** with a methoxy group (**52e**) afforded a 30-fold increase in human recombinant B_2 affinity, while larger substituents, ethoxy (**52g**), methoxymethyl (**52h**), and ethoxycarbonylmethyl (**52i**) groups, resulted in a severe loss of activity. Furthermore, the 2,6-dichlorophenyl group of **52e** could be replaced by the 2,6-dimethylphenyl group (**52f**) with only a slight loss of binding affinity and improved in vivo activity after oral administration. Substitution of the methoxy group of **52f** with an electron-withdrawing acetyl group (**52j**) decreased the binding affinity much more severely for guinea pig ileum B_2 receptors than for human recombinant B_2 receptors. Replacement of the oxygen atom of the methoxy group with other heteroatoms gave much less potent compounds: i.e., the sulfide derivative **52k** and the amino derivatives **52l**,**m**. It has thus been revealed that the substituents on the imidazo[1,2-*a*]pyridine[21] and benzimidazole rings make important contributions to binding to B_2 receptors. It is interesting that the optimum combinations of substituents on both rings have similar steric requirements, even though they have different electrostatic features.

With the potent benzimidazole B_2 antagonists **52e**,**f** (with nanomolar IC_{50}'s) in hand, we next investigated improvement of their in vivo activities by modifying the terminal phenyl moiety (Table 3). *N*-Methylation of the methylcarbamoyl group of **52e** (**72**) afforded a remarkable increase in inhibitory activity against BK-induced bronchoconstriction, while the binding affinity for human recombinant B_2 receptors was diminished. Replacement of the *N*-methylcarbamoylphenyl moiety of **52e**,**f** with the acetamidopyridyl group (**73a**,**b**) significantly improved in vivo B_2 antagonistic activity again, retaining potent binding affinities for both human and guinea pig B_2 receptors. In particular, compound **73b** when dosed orally at 0.32 mg/kg inhibited BK-induced bronchoconstriction with an inhibitory value of 75.2%.

As shown in Table 4, we attempted to optimize quinoxaline derivatives by introducing several combinations of R^1, R^2, and X which had been proven to afford potent binding affinities for human recombinant B_2 receptors in the imidazo[1,2-*a*]pyridine and benzimidazole series. However, most of the quinoxaline derivatives suffered from a more severe specific difference than the former series. They did not exhibit potent binding affinities for human recombinant B_2 receptors, except for **57a** and **76a**, which failed to show significant in vivo antagonistic activities at 1 mg/kg po. This trend was also observed in the quinazoline series (data not shown). The results indicate that introduction of the additional nitrogen atom at the 3- or 4-position within the quinoline ring is unfavorable for in vitro B_2 binding affinity and in vivo antagonistic activity.

Table 5 shows optimization of the quinoline series. Consistent with the previous study on the imidazo[1,2-*a*]pyridine series, **81a**,**b**, which do not possess a carbamoyl or amide substituent on the terminal aromatic ring, showed much lower binding affinities for human recombinant B_2 receptors compared with those for

Table 1. Replacement of the Imidazo[1,2-*a*]pyridine Ring



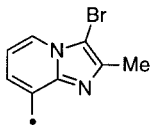
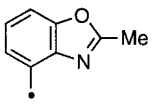
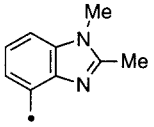
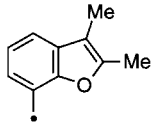
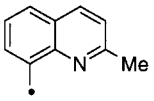
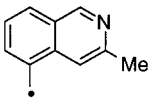
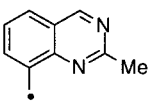
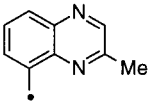
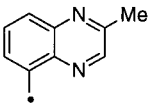
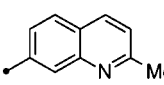
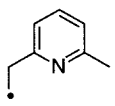
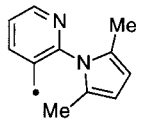
compd	R	*n*	in vitro IC_{50} (nM) guinea pig ileum[a]	human A-431 cell[b]	human recombinant[c]	synth method[d]	mp (°C)	formula[e]
4b		1	0.50	1.5	9.0	ref[23]	amorphous	$C_{29}H_{26}BrCl_2N_5O_4$•HCl
51		0	380	860	1100	A	amorphous	$C_{29}H_{26}Cl_2N_4O_5$
52a		0	9.1	43	67	A	amorphous	$C_{30}H_{29}Cl_2N_5O_4$
53		0	23	NT[f]	270	A	237–238	$C_{31}H_{29}Cl_2N_3O_5$
54a		1	0.51	1.3	1.1	B	160–165	$C_{31}H_{28}Cl_2N_4O_4$•HCl•H_2O
55		0	>10000	>10000	NT	A	amorphous	$C_{31}H_{28}Cl_2N_4O_4$
56		0	110	59	56	A	amorphous	$C_{30}H_{27}Cl_2N_5O_4$
57a		1	1.0	3.1	5.2	B	amorphous	$C_{30}H_{27}Cl_2N_5O_4$•HCl
58		0	170	4300	2900	A	amorphous	$C_{30}H_{27}Cl_2N_5O_4$
59		0	>10000	>10000	NT	A	amorphous	$C_{31}H_{28}Cl_2N_4O_4$
60		0	>10000	>10000	>10000	A	amorphous	$C_{28}H_{28}Cl_2N_4O_4$

Table 1. (Continued)

compd	R	*n*	in vitro IC_{50} (nM) guinea pig ileum[a]	human A-431 cell[b]	human recombinant[c]	synth method[d]	mp (°C)	formula[e]
61		0	350	3500	2000	A	amorphous	$C_{32}H_{31}Cl_2N_5O_4$
4a		1	0.66	1.4	2.2	ref[23]	amorphous	$C_{30}H_{28}BrCl_2N_5O_4 \cdot HCl \cdot H_2O$
Icatibant			0.09	3.3	0.49			

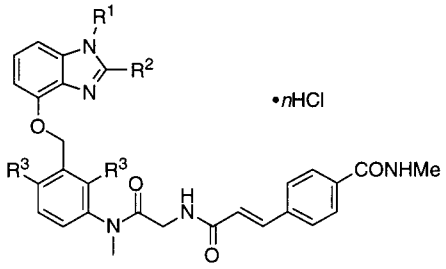
[a] Concentration required to inhibit specific binding of [^{3}H]BK (0.06 nM) to B_2 receptors in guinea pig ileum membrane preparations by 50%. Values are expressed as the average of at least three determinations, with variation in individual values of <15%. See the Experimental Section for further 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] Concentration required to inhibit specific binding of [^{3}H]BK (1.0 nM) to human B_2 receptors which was expressed in CHO (Chinese hamster ovary) cells by 50%. Values are expressed as the average of at least three determinations, with variation in individual values of <15%. See the Experimental Section for further details. [d] For details, see the Experimental Section. [e] Analyses for C, H, and N are within ±0.4% of the expected value for the formula. [f] NT, not tested.

guinea pig ileum B_2 receptors. On the basis of the SAR discussed above, further promising chemical modifications were performed. Most of the derivatives displayed potent activities both in vitro and in vivo. Comparison of the binding affinities of **54a** and **84** for human A-431 B_2 receptors also supported our initial postulation that para substitution at the terminal phenyl ring would be suitable because of topological similarity with meta-substituted phenyl ureas.[23] Generally, 2,6-dimethyl derivatives (**54b**,**d**, **88c**, **90b**,**d**) are somewhat less potent than the corresponding 2,6-dichloro congeners (**54a**,**c**, **87b**, **90a**,**c**) in B_2 binding affinities, while **54b** and **90b** showed superior in vivo activities to **54a** and **90a**, respectively. Replacement of the phenyl ring of the cinnamoyl moiety (**54a**,**b**, **81a**, **90a**,**b**) with a pyridine ring (**54c**,**d**, **81b**, **90c**,**d**) also resulted in decreased binding activities for human recombinant B_2 receptors with one exception (**88a** vs **87b**). **87b** afforded highly potent B_2 binding affinities for both guinea pig ileum (IC_{50} = 0.46 nM) and human recombinant (IC_{50} = 1.4 nM) B_2 receptors and had the best in vivo B_2 antagonistic activity among the non-peptide antagonists evaluated so far, with an ED_{50} value of 0.075 mg/kg by oral administration. This well-balanced in vitro and in vivo pharmacological profile led us to select compound **87b** as the second clinical candidate for treatment of various inflammatory diseases such as rhinitis and asthma. We next investigated the SAR of the terminal substituent in more detail. Replacement of the terminal methyl group of **54a** with a phenyl ring (**91**) caused a 1 order of magnitude decrease in both human and guinea pig binding affinities. However, introduction of a nitrogen atom to the terminal phenyl ring of **81** led to discovery of a new pharmacophore. The 2-pyridyl derivative **92a** showed severalfold weaker binding affinities in both humans and guinea pigs than **91**. The 3-pyridyl derivative **93b** regained the activity in guinea pigs to the same level as **54a**, and its binding affinity for human recombinant B_2 receptors was comparable to that of **91**. On the other hand, incorporation of a 4-pyridyl ring afforded **93c** with low-nanomolar IC_{50}'s for both human recombinant and guinea pig B_2 receptors. Although **93c** failed to show significant in vivo antagonistic activity at 1 mg/kg po, the pyridyl acrylamide analogue **95**, which retained human B_2 affinity, exhibited significantly improved in vivo activity despite its 14-fold less potent B_2 binding activity for guinea pigs compared with **93c**. These results indicate that the 4-nitrogen atom of **93c** and **95** interacts with both human and guinea pig B_2 receptors. To optimize the geometry of this new pharmacophore, we modified the junction between the two pyridine rings. The inverse amide **98** improved guinea pig B_2 affinity and in vivo activity. Furthermore, the (*E*)-ethenyl derivative **99** afforded subnanomolar IC_{50}'s for both human and guinea pig B_2 receptors along with further enhancement of in vivo antagonistic activity. In the human recombinant B_2 receptor binding assay, **99** was as potent as the second-generation peptide antagonist Icatibant. It is also noteworthy that **99** had high enough water solubility to explore potential new indications for which intravenous administration is required.

Conclusion

In this study, employing **4a** as a lead compound, we determined that the imidazo[1,2-*a*]pyridine moiety of the basic framework could be successfully replaced by several heterocyclic bioisosteres. Among them, the 1-methyl-2-methoxy-1*H*-benzimidazole and 2-methylquinoline derivatives showed potent B_2 binding affinities against both human and guinea pig B_2 receptors. These results indicate that the 1-nitrogen atom of the heterocycles makes an essential electrostatic interaction with B_2 receptors, while the 4-nitrogen atom is unnecessary. Moreover, the 3-substituent which increases affinity in the 5−6 condensed-ring systems is not necessary in the 6−6 condensed-ring systems. We also found that replacement of the 2,6-dichlorophenyl moiety with 2,6-dimethylphenyl group generally affords improvement of in vivo antagonistic activities along with slightly decreased binding activities. As the results of extensive optimization of these new heterocyclic series, we finally identified **87b** as our second clinical candi-

Table 2. Introduction of Substituents at the 1,2-Positions of the Benzimidazole Moiety



compd	R^1	R^2	R^3	n	in vitro IC_{50} (nM) guinea pig ileum[a]	in vitro IC_{50} (nM) human recombinant[b]	in vivo % inhibn[c] 1 mg/kg, po	synth method[d]	mp (°C)	formula[e]
52a	Me	Me	Cl	0	9.1	67	NT[f]	A	amorphous	$C_{30}H_{29}Cl_2N_5O_4$
52b	Et	Me	Cl	0	8.9	48	NT	A	amorphous	$C_{31}H_{31}Cl_2N_5O_4$
64c	Me	Et	Cl	1	8.7	22	31.7±10.6	B	amorphous	$C_{31}H_{31}Cl_2N_5O_4$•HCl
52d	Me	Ph	Cl	0	510	>1000	NT	A	amorphous	$C_{35}H_{31}Cl_2N_5O_4$
52e	Me	OMe	Cl	0	0.83	2.2	21.7±13.9	A	244–249	$C_{30}H_{29}Cl_2N_5O_5$
52f	Me	OMe	Me	0	1.6	7.2	59.7±9.4**	A	amorphous	$C_{32}H_{35}N_5O_5$
52g	Me	OEt	Me	0	90	730	NT	A	226–231	$C_{33}H_{37}N_5O_5$
52h	Me	CH_2OMe	Me	0	1000	9100	NT	A	232–235	$C_{33}H_{37}N_5O_5$
52i	Me	CH_2CO_2Et	Me	0	1100	8400	NT	A	132–140	$C_{35}H_{39}N_5O_6$
52j	Me	Ac	Me	0	200	94	NT	A	234–236	$C_{33}H_{35}N_5O_5$
52k	Me	SMe	Me	0	9.5	79	NT	Ex[g]	224–225	$C_{32}H_{35}N_5O_4S$
52l	Me	NHMe	Me	0	94	670	NT	A	amorphous	$C_{32}H_{36}N_6O_4$
52m	Me	NMe_2	Me	0	38	160	NT	A	amorphous	$C_{33}H_{38}N_6O_4$

[a,b,d–f] See corresponding footnotes in Table 1. [c] BK (5 μg/kg) was administered intravenously to anesthetized guinea pigs, and bronchoconstriction induced by the BK administration was measured by the modified Konzett and Rösseler method[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. [g] Ex, experimental procedure described.

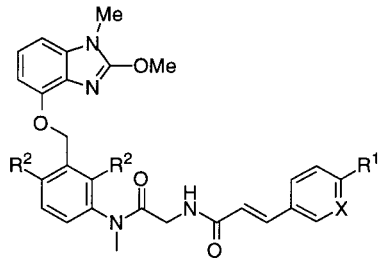
date. Since **87b** exhibited highly potent B_2 binding affinities for both humans and guinea pigs, with IC_{50}'s of 1.4 and 0.46 nM, respectively, and excellent in vivo functional antagonistic activity with an ED_{50} value of 0.075 mg/kg (po), it is expected to be the first member of a novel class of drug for the treatment of various inflammatory diseases. Further modifications of the terminal substituents enabled us to discover a novel pharmacophore, leading to the discovery of **99** which exhibited the highest binding affinity for human recombinant B_2 receptors among the non-peptide B_2 antagonists evaluated so far. The IC_{50} value of this compound (0.51 nM) is comparable to that of Icatibant (0.49 nM). **99** also showed highly potent in vitro and in vivo activity in guinea pigs and good water solubility to enable examination of new indications for which intravenous administration is required.

Experimental Section

Chemistry. Melting points were determined on a Mel-Temp (Mitamura Riken Kogyo, Japan) and are uncorrected. 300-MHz Proton NMR spectra were recorded on a Varian Gemini 300 spectrometer, and shifts are expressed in δ (ppm) with TMS as internal standard. Mass spectra were recorded with a VG (Fisons) ZAB-SE (FAB) or Micromass Platform (ESI) system. IR spectra were recorded 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.

Ethyl 3-Methoxy-2-nitrophenylcarbamate (6). To a suspension of **5** (10.0 g, 50.7 mmol) in dry benzene (100 mL) were added triethylamine (7.07 mL, 50.7 mmol) and diphenyl phosphorazidate (14.0 g, 50.7 mmol) at ambient temperature. The mixture was refluxed for 40 min, and dry EtOH (3.28 mL, 55.8 mmol) was added therein. After 40 min, the cooled mixture was concentrated in vacuo. To this mixture was added AcOEt (100 mL), and the precipitate was removed by filtration with Celite. The filtrate was washed with saturated

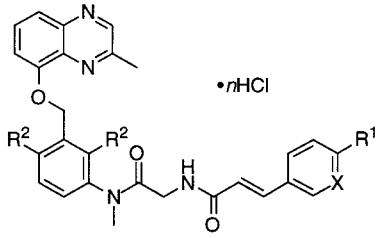
Table 3. Optimization of the Benzimidazole Series



compd	R^1	R^2	X	in vitro IC_{50} (nM) guinea pig ileum[a]	in vitro IC_{50} (nM) human recombinant[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]
52e	CONHMe	Cl	CH	0.83	2.2	NT[f]	21.7±13.9	A	244–249	$C_{30}H_{29}Cl_2N_5O$
52f	CONHMe	Me	CH	1.6	7.2	NT	59.7±9.4**	A	amorphous	$C_{32}H_{35}N_5O_5$
72	$CONMe_2$	Cl	CH	0.94	14	NT	87.7±1.2***	E	amorphous	$C_{31}H_{31}Cl_2N_5O$
73a	NHAc	Cl	N	0.65	4.2	NT	55.4±16.8*	E	amorphous	$C_{29}H_{28}Cl_2N_6O$
73b	NHAc	Me	N	0.94	8.5	75.2±0.6**	98.4±0.7***	A	amorphous	$C_{31}H_{34}N_6O_5$

[a,b,d–f] See corresponding footnotes in Table 1. [c] See corresponding footnote in Table 2.

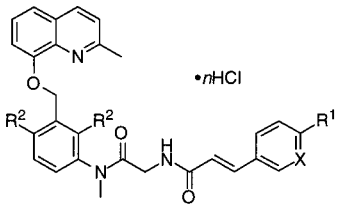
Table 4. Optimization of the Quinoxaline Series



compd	R^1	R^2	X	*n*	in vitro IC_{50} (nM) guinea pig ileum[a]	in vitro IC_{50} (nM) human recombinant[b]	in vivo % inhibin[c] 1 mg/kg, po	synth method[d]	mp (°C)	formula[e]
57a	CONHMe	Cl	CH	1	1.0	5.2	22.2±21.2	B	amorphous	$C_{30}H_{27}Cl_2N_5O_4$•HCl
63b	CONHMe	Me	CH	0	3.5	17	NT[f]	A	amorphous	$C_{32}H_{33}N_5O_4$
63c	CONHMe	Me	N	0	18	590	NT	C	amorphous	$C_{31}H_{32}N_6O_4$
74a	$CONMe_2$	Cl	CH	0	1.9	44	NT	E	110–114	$C_{31}H_{29}Cl_2N_5O_4$
74b	$CONMe_2$	Me	CH	0	4.1	130	NT	E	amorphous	$C_{33}H_{35}N_5O_4$
75a		Cl	CH	0	1.1	26	NT	E	amorphous	$C_{32}H_{29}Cl_2N_5O_4$
75b		Me	CH	0	2.4	46	NT	E	amorphous	$C_{34}H_{35}N_5O_4$
76a	NHAc	Cl	CH	1	2.4	2.5	28.6±12.4	B	amorphous	$C_{30}H_{27}Cl_2N_5O_4$•HCl
77b	NHAc	Me	N	0	48	130	NT	E	amorphous	$C_{31}H_{32}N_6O_4$

[a,b,d–f] See corresponding footnotes in Table 1. [c] See corresponding footnote in Table 2.

Table 5. Optimization of the Quinoline Series



compd	R^1	R^2	X	*n*	in vitro IC_{50} (nM) guinea pig ileum[a]	in vitro IC_{50} (nM) human recombinant[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]
54a	CONHMe	Cl	CH	1	0.51	1.1 (1.3)[g]	67.4±6.5***	81.9±8.0***	B	160–165	$C_{33}H_{34}N_4O_4$•HCl
81a	H	Cl	CH	1	0.69	340	NT[f]	25.2±32.0*	B	amorphous	$C_{29}H_{25}Cl_2N_3O_3$•HCl
81b	H	Cl	N	2	0.79	470	63.7±3.2*	97.2±0.4*	B	amorphous	$C_{28}H_{24}Cl_2N_4O_3$•HCl
84	H	Cl	C-$CONMe_2$	0	1.1	(14)[g]	NT	NT	C	amorphous	$C_{31}H_{28}Cl_2N_4O_4$
54b	CONHMe	Me	CH	1	0.97	4.8	79.0±8.3*	90.5±3.9***	B	amorphous	$C_{33}H_{34}N_4O_4$•HCl
54c	CONHMe	Cl	N	2	0.68	4.6	68.4±1.0	93.9±1.3**	B	amorphous	$C_{30}H_{27}Cl_2N_5O_4$•2HCl
54d	CONHMe	Me	N	2	2.2	58	NT	NT	B	amorphous	$C_{32}H_{33}N_5O_4$•2HCl
86	$CONMe_2$	Cl	CH	1	1.3	3.9	50.8±20.6	89.7±0.5**	B	amorphous	$C_{32}H_{30}Cl_2N_4O_4$•HCl
88a	NHAc	Cl	CH	1	0.50	1.9	33.2±9.6	80.0±5.4***	B	amorphous	$C_{31}H_{28}Cl_2N_4O_4$•HCl
87b	NHAc	Cl	N	0	0.46	1.4	73.2±7.7***	94.4±3.0**	E	146–150	$C_{30}H_{27}Cl_2N_5O_4$•$2H_2O$
88c	NHAc	Me	N	2	0.82	8.5	55.5±14.6**	98.2±0.8***	B	amorphous	$C_{32}H_{33}N_5O_4$•2HCl
90a		Cl	CH	1	0.55	3.2	44.7±7.1**	85.3±5.1*	B	amorphous	$C_{33}H_{30}Cl_2N_4O_4$•HCl
90b		Me	CH	1	0.47	9.3	75.7±1.7**	94.9±0.8***	B	amorphous	$C_{35}H_{36}N_4O_4$•HCl
90c		Cl	N	2	0.13	5.2	68.6±5.4*	89.9±6.2***	B	amorphous	$C_{32}H_{29}Cl_2N_5O_4$•2HCl
90d		Me	N	2	1.4	39	NT	NT	B	amorphous	$C_{34}H_{35}N_5O_4$•2HCl
91	CONHPh	Cl	CH	0	6.3	11	NT	NT	E	amorphous	$C_{36}H_{30}Cl_2N_4O_4$
92a	CONH-2-Py	Cl	CH	0	11	30	NT	NT	E	amorphous	$C_{35}H_{29}Cl_2N_5O_4$
93b	CONH-3-Py	Cl	CH	2	0.62	7.5	NT	46.8±9.7*	B	amorphous	$C_{35}H_{29}Cl_2N_5O_4$•2HCl
93c	CONH-4-Py	Cl	CH	2	1.1	1.8	NT	29.5±17.0	B	amorphous	$C_{35}H_{29}Cl_2N_5O_4$•2HCl
95	CONH-4-Py	Cl	N	3	15	2.5	NT	60.0±6.2**	B	amorphous	$C_{34}H_{28}Cl_2N_6O_4$•3HCl
98	NHCO-4-Py	Cl	N	3	3.3	2.8	NT	68.8±3.9**	B	amorphous	$C_{34}H_{28}Cl_2N_6O_4$•3HCl
99	(*E*)-CH=CH-4-Py	Cl	N	0	0.76	0.51	43.6±12.9	87.3±4.3**	E	amorphous	$C_{35}H_{29}Cl_2N_5O_3$
Icatibant					0.09	0.49					

[a,b,d–f] See corresponding footnotes in Table 1. [c] See corresponding footnote in Table 2. [g] IC_{50} for inhibition of specific binding of [^{3}H]BK (1.2 nM) to human A-431 cells.

aqueous sodium bicarbonate, water, and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel chromatography (hexane−AcOEt, 4:1) followed by crystallization from hexane to give **6** (7.23 g, 59.4%) as pale-yellow

crystals: mp 131−132 °C; ^{1}H NMR ($CDCl_3$) δ 1.31 (3H, d, J = 7.5 Hz), 3.90 (3H, s), 4.22 (2H, q, J = 7.5 Hz), 6.73 (1H, d, J = 7.5 Hz), 7.41 (1H, t, J = 7.5 Hz), 7.71 (1H, br s), 7.78 (1H, d, J = 7.5 Hz). Anal. ($C_{10}H_{12}N_2O_5$) C, H, N.

Compound **13** was prepared using a similar procedure to that described for **6**.

3-Methoxy-1-methyl-1,2-phenylenediamine (7). To a suspension of lithium aluminum hydride (316 mg, 8.32 mmol) in dry THF (10 mL) was added **6** (1.00 g, 4.16 mmol) in portions in an ice−water bath under nitrogen. After 30 min, the reaction mixture was stirred at ambient temperature for 1 h. To the mixture was added water below 10 °C. The precipitate was removed by vacuum filtration through Celite and washed with CH_2Cl_2. The filtrate and washings were combined, dried, and evaporated in vacuo. The residue was purified by flash silica gel chromatography (hexane−AcOEt, 3:1) followed by crystallization from hexane to give **7** (190 mg, 29.9%) as brown crystals: mp 60−61 °C; ^{1}H NMR ($CDCl_3$) δ 2.88 (3H, s), 3.40 (2H, br s), 3.84 (3H, s), 6.38 (1H, d, J = 7.5 Hz), 6.42 (1H, d, J = 7.5 Hz), 6.81 (1H, t, J = 7.5 Hz); MS (FAB) m/z 152 (M + 1). Anal. ($C_8H_{12}N_2O$) C, H, N.

Compound **102** was prepared using a similar procedure to that used for **7**.

1,2-Dimethyl-4-methoxy-1*H*-benzimidazole (8). A suspension of **7** (671 mg, 4.41 mmol) and AcOH (265 mg, 4.41 mmol) in 4 N HCl (3.5 mL) was refluxed for 4 h. After cooling, the reaction mixture was adjusted to pH 8 with 28% ammonium hydroxide solution. The mixture was extracted with CH_2Cl_2, and the organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel chromatography (CH_2Cl_2−MeOH, 40:1) followed by crystallization from isopropyl ether to give **8** (680 mg, 87.5%) as pale-brown crystals: mp 123−124 °C; ^{1}H NMR ($CDCl_3$) δ 2.59 (3H, s), 3.70 (3H, s), 4.00 (3H, s), 6.67 (1H, d, J = 7.5 Hz), 6.89 (1H, d, J = 7.5 Hz), 7.17 (1H, t, J = 7.5 Hz); MS (FAB) m/z 177 (M + 1). Anal. ($C_{10}H_{12}N_2O$) C, H, N.

Compound **20e** was prepared using a similar procedure to that used for **8**.

1,2-Dimethyl-4-hydroxy-1*H*-benzimidazole (9). To a solution of **8** (560 mg, 3.18 mmol) in dry CH_2Cl_2 (2 mL) was added 1 M boron tribromide in CH_2Cl_2 solution (6.36 mL) at 3−5 °C under nitrogen. After 30 min, the reaction mixture was stirred at ambient temperature for 2 h and refluxed for 14 h. The cooled mixture was adjusted to pH 7 with saturated sodium hydrogen carbonate and extracted with CH_2Cl_2 (2×). The organic layer was dried and evaporated in vacuo. The residue was crystallized from AcOEt to give **9** (297 mg, 57.6%) as colorless crystals: mp 243−245 °C; ^{1}H NMR ($CDCl_3$) δ 2.64 (3H, s), 3.69 (2H, br s), 4.00 (3H, s), 6.81 (2H, d, J = 7.5 Hz), 7.17 (1H, t, J = 7.5 Hz); MS (FAB) m/z 163 (M + 1). Anal. ($C_9H_{10}N_2O$) C, H, N.

Compounds **32** and **38** were prepared using a similar procedure to that used for **9**.

Benzyl 3-Benzyloxy-2-nitrobenzene (11). To a mixture of **10** (500 mg, 2.73 mmol) and potassium carbonate (1.13 g, 8.19 mmol) in dry DMF (5 mL) was added benzyl bromide (1.12 g, 6.55 mmol) at ambient temperature. After 14 h, the mixture was poured into water and extracted with AcOEt twice. The organic layer was washed with water (3×) and brine, dried, and evaporated in vacuo. The residue was purified by flash column chromatography (hexane−AcOEt, 3:1) followed by crystallization from isopropyl ether to give **11** (676 mg, 92.2%) as colorless crystals: mp 83−85 °C; ^{1}H NMR ($CDCl_3$) δ 5.20 (2H, s), 5.32 (2H, s), 7.22−7.46 (12H, m), 7.61 (1H, d, J = 8 Hz). Anal. ($C_{21}H_{17}NO_5$) C, H, N.

3-Benzyloxy-1-methyl-2-nitroaniline (15a). A mixture of **14a** (5.80 g, 16.2 mmol) in 4 N hydrogen chloride in AcOEt (29 mL, 116 mmol) was stirred in an ice-cooled bath for 30 min. The reaction mixture was evaporated in vacuo. To the residue was added saturated aqueous sodium bicarbonate, and then the mixture was extracted with AcOEt. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The resulting residue was then recrystallized from hexane−AcOEt to give **15a** (3.51 g, 84.0%) as orange crystals: mp 80−81 °C; ^{1}H NMR ($CDCl_3$) δ 2.91 (3H, d, J = 7.5 Hz), 5.15 (2H, s), 6.21 (1H, br d, J = 7.5 Hz), 6.33 (1H, d, J = 7.5 Hz), 6.38 (1H, d, J = 7.5 Hz), 7.25 (1H, t, J = 7.5 Hz), 7.29−7.49 (5H, m). Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

Compound **15b** was prepared using a similar procedure to that used for **15a**.

3-Benzyloxy-1-(*N*-methyl-*N*-propanoyl)-2-nitroaniline (16a). To a solution of **15a** (1.70 g, 6.58 mmol) in *N*,*N*-dimethylaniline (5 mL) was added propionyl chloride (1.22 g, 13.2 mmol) at ambient temperature under nitrogen. The reaction mixture was heated at 90 °C for 2 h. After cooling, the mixture was poured into water and extracted with AcOEt. The organic layer was washed with water, saturated aqueous sodium bicarbonate, and brine, dried, and evaporated in vacuo. The resulting residue was purified by flash silica gel column chromatography (hexane−AcOEt, 3:1) to give **16a** (1.76 g, 85.1%) as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.91 (1H, t, J = 7.5 Hz), 1.16 (1H, t, J = 7.5 Hz), 1.95−2.22 (1.3H, m), 2.33−2.60 (0.7H, m), 3.15−3.27 (3H, m), 5.16−5.25 (2H, m), 6.89 (1H, m), 7.15 (1H, m), 7.30−7.53 (6H, m). Anal. ($C_{17}H_{18}N_2O_4$) C, H, N.

Compounds **16b**−**e** were prepared using a similar procedure to that used for **16a**.

4-Benzyloxy-2-ethyl-1-methyl-1*H*-benzimidazole (17a). A suspension of **16a** (1.60 g, 5.09 mmol) and iron (powder, 2.84 g, 50.9 mmol) in a mixture of AcOH (13 mL) and EtOH (6.4 mL) was refluxed for 3 h. After cooling, the mixture was filtered through Celite, and the filtrate was evaporated in vacuo. The residue was neutralized with saturated aqueous sodium bicarbonate and extracted with AcOEt twice. The organic layer was washed with saturated aqueous sodium bicarbonate, water, and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel column chromatography (hexane−AcOEt, 3:1) followed by crystallization from hexane to give **17a** (481 mg, 35.2%) as colorless crystals: mp 95−96 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (3H, t, J = 7.5 Hz), 2.95 (2H, q, J = 7.5 Hz), 3.70 (3H, s), 5.39 (2H, s), 6.65 (1H, d, J = 7.5 Hz), 6.89 (1H, d, J = 7.5 Hz), 7.09 (1H, t, J = 7.5 Hz), 7.25−7.40 (3H, m), 7.51 (2H, br d, J = 7.5 Hz). Anal. ($C_{17}H_{18}N_2O$) C, H, N.

Compounds **17b**−**e** were prepared using a similar procedure to that used for **17a**.

2-Ethyl-4-hydroxy-1-methyl-1*H*-benzimidazole (18a). A mixture of **17a** (500 mg, 1.88 mmol) and 10% palladium on carbon (50 mg) in MeOH (5 mL) was stirred at ambient temperature under hydrogen for 2 h. The reaction mixture was filtered through Celite and evaporated in vacuo, and the residue was crystallized from isopropyl ether to give **18a** (285 mg, 78.9%) as colorless crystals: mp 233−235 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (3H, t, J = 7.5 Hz), 2.98 (2H, q, J = 7.5 Hz), 3.70 (3H, s), 6.77−6.86 (2H, m), 7.15 (1H, t, J = 8 Hz), 6.89 (1H, d, J = 7.5 Hz), 7.09 (1H, t, J = 7.5 Hz), 7.25−7.40 (3H, m), 7.51 (2H, br d, J = 7.5 Hz); MS (FAB) m/z 177 (M + 1). Anal. ($C_{10}H_{12}N_2O$) C, H, N.

Compounds **18b**−**e**, **21a**−**d**,**f**, and **22** were prepared using a similar procedure to that used for **18a**.

3-Benzyloxy-1-methyl-1,2-phenylenediamine (19). To a mixture of **15a** (453 mg, 1.76 mmol), iron(III) chloride hexahydrate (14 mg), and carbon (14 mg) in 80% aqueous MeOH (6.8 mL) was added dropwise hydrazine monohydrate (256 mg, 4.16 mmol) at 70 °C. The reaction mixture was stirred at 70 °C for 5 h, cooled to ambient temperature, and filtered through Celite. The filtrate was concentrated in vacuo and the residue partitioned between AcOEt and saturated aqueous sodium bicarbonate. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel column chromatography (hexane−AcOEt, 3:1) followed by crystallization from hexane−isopropyl ether to give **19** (349 mg, 87.1%) as colorless crystals: mp 79−81 °C; ^{1}H NMR ($CDCl_3$) δ 2.87 (3H, s), 3.43 (3H, br s), 5.07 (2H, s), 6.40 (1H, d, J = 7.5 Hz), 6.49 (1H, d, J = 7.5 Hz), 6.80 (1H, t, J = 7.5 Hz), 7.29−7.47 (5H, m). Anal. ($C_{14}H_{16}N_2O$) C, H, N.

Compounds **44a**,**b** were prepared using a similar procedure to that used for **19**.

4-Benzyloxy-2-methoxy-1-methyl-1*H*-benzimidazole (20a). To a stirred solution of **19** (319 mg, 1.40 mmol) in AcOH (3.2 mL) was added tetramethyl orthocarbonate (223 mg, 1.68 mmol) at ambient temperature. After 4 h, the reaction mixture was concentrated in vacuo. The residue was partitioned between AcOEt and saturated aqueous sodium bicarbonate. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was then purified by flash silica gel column chromatography (hexane−AcOEt, 3:1) followed by crystallization from hexane−isopropyl ether to give **20a** (273 mg, 73.0%) as colorless crystals: mp 100−102 °C; ^{1}H NMR ($CDCl_3$) δ 3.53 (3H, s), 4.22 (3H, s), 5.40 (2H, s), 6.63 (1H, d, J = 7.5 Hz), 6.77 (1H, d, J = 7.5 Hz), 6.99 (1H, t, J = 7.5 Hz), 7.22−7.41 (3H, m), 7.45−7.52 (3H, m); MS (ESI) m/z 269 (M + 1). Anal. ($C_{16}H_{16}N_2O_2$) C, H, N.

Compounds **20b** was prepared using a similar procedure to that used for **20a**.

4-Benzyloxy-1-methyl-2-methylamino-1*H*-benzimidazole (20c). A mixture of **19** (200 mg, 0.877 mmol) and methyl isothiocyanate (71 mg, 0.964 mmol) in dry THF (2 mL) was stirred for 2 days at ambient temperature under nitrogen. The solvent was removed in vacuo, and the residue was dissolved in MeCN (2 mL). To the solution was added methyl iodide (149 mg, 1.05 mmol) in an ice−water bath. The mixture was stirred for 5 h at the same temperature and concentrated in vacuo. The residue was crystallized from AcOEt and then recrystallized from MeCN to give **20c** (148 mg, 63.1%) as colorless needles: mp 213−214 °C; ^{1}H NMR ($CDCl_3$) δ 3.12 (3H, s), 3.80 (3H, s), 5.20 (2H, s), 6.87 (2H, d, J = 7.5 Hz), 7.22 (1H, t, J = 7.5 Hz), 7.34−7.51 (5H, m); MS (ESI) m/z 178 (M + 1). Anal. ($C_{16}H_{17}N_3O$) C, H, N.

2-Acetyl-4-benzyloxy-1-methyl-1*H*-benzimidazole (20f). To a solution of **20e** (87 mg, 0.308 mmol) in CH_2Cl_2 (3 mL) was added manganese(IV) oxide (870 mg, 10.0 mmol) in an ice−water bath under nitrogen. The reaction mixture was stirred in an ice−water bath for 30 min and at ambient temperature for 2 h. The mixture was purified directly by flash silica gel column chromatography (hexane−AcOEt, 1:3) followed by crystallization from isopropyl ether to give **20f** (74 mg, 85.9%) as colorless crystals: mp 102−104 °C; ^{1}H NMR ($CDCl_3$) δ 2.88 (3H, s), 4.11 (3H, s), 5.46 (2H, s), 6.72 (1H, d, J = 8.5 Hz), 7.00 (1H, d, J = 8.5 Hz), 7.23−7.41 (4H, m), 7.52 (2H, d, J = 7.5 Hz); MS (ESI) m/z 281 (M + 1). Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

4-[[2,6-Dimethyl-3-[*N*-methyl-*N*-[(*E*)-4-(*N*-methylcarbamoyl)cinnamamidoacetyl]amino]benzyl]oxy]-2-mercapto-1-methyl-1*H*-benzimidazole (24). To a solution of **23** (150 mg, 0.283 mmol) in dry THF (3 mL) was added 1,1′-thiocarbonyldiimidazole (76 mg, 0.425 mmol) at ambient temperature under nitrogen. After 7 h, water was added and the reaction mixture extracted with $CHCl_3$. The organic layer was washed with water and brine, dried, and then evaporated in vacuo. The residue was purified by flash silica gel column chromatography ($CHCl_3$−MeOH, 30:1) followed by crystallization from diethyl ether to give **24** (130 mg, 80.6%) as pale-brown crystals: mp 279−292 °C; ^{1}H NMR (DMSO-d_6) δ 2.26 (3H, s), 2.42 (3H, s), 2.79 (3H, d, J = 5 Hz), 3.11 (3H, s), 3.49 (1H, dd, J = 17, 5 Hz), 3.63 (3H, s), 3.71 (1H, dd, J = 17, 5 Hz), 5.23 (1H, d, J = 11 Hz), 5.30 (1H, d, J = 11 Hz), 6.90 (1H, d, J = 15 Hz), 7.03 (1H, d, J = 8 Hz), 7.09 (1H, d, J = 8 Hz), 7.20 (1H, t, J = 8 Hz), 7.25 (1H, d, J = 8 Hz), 7.31 (1H, d, J = 8 Hz), 7.44 (1H, d, J = 15 Hz), 7.64 (2H, d, J = 8 Hz), 7.85 (2H, d, J = 8 Hz), 8.27 (1H, t, J = 5 Hz), 8.48 (1H, q, J = 5 Hz); MS (ESI) m/z 572 (M + 1). Anal. ($C_{31}H_{33}N_5O_4S$) C, H, N.

4-[[2,6-Dimethyl-3-[*N*-methyl-*N*-[(*E*)-4-(*N*-methylcarbamoyl)cinnamamidoacetyl]amino]benzyl]oxy]-1-methyl-2-methylthio-1*H*-benzimidazole (52k). To a mixture of **24** (120 mg, 0.210 mmol) and potassium carbonate (44 mg, 0.315 mmol) in dry DMF (1.2 mL) was added methyl iodide (31 mg, 0.221 mmol) at ambient temperature. After 16 h, the mixture was poured into water and extracted with $CHCl_3$. The organic layer was washed with water (3×) and brine, dried, and evaporated in vacuo. The residue was purified by flash column chromatography (AcOEt−MeOH, 20:1) followed by crystallization from diethyl ether to give **52k** (130 mg, 80.6%) as colorless crystals: mp 224−225 °C; ^{1}H NMR (DMSO-d_6) δ 2.30 (3H, s), 2.43 (3H, s), 2.67 (3H, s), 2.77 (3H, d, J = 5 Hz), 3.09 (3H, s), 3.48 (1H, dd, J = 17, 5 Hz), 3.63 (3H, s), 3.64 (1H, dd, J = 17, 5 Hz), 5.35 (2H, s), 6.87 (1H, d, J = 15 Hz), 6.90 (1H, m), 7.07−7.15 (2H, m), 7.23 (1H, d, J = 8 Hz), 7.30 (1H, d, J = 8 Hz), 7.40 (1H, t, J = 15 Hz), 7.61 (2H, d, J = 8 Hz), 7.83 (2H, d, J = 8 Hz), 8.23 (1H, t, J = 5 Hz), 8.47 (1H, q, J = 5 Hz); MS (ESI) m/z 586 (M + 1). Anal. ($C_{32}H_{35}N_5O_4S$) C, H, N.

2-Methylisoquinoline-5-sulfonic Acid (26). Compounds **26** and **27** were prepared by a similar method of Georgian et al.[25] To concentrated sulfuric acid (3.4 mL) were added **25** (8.83 g, 61.7 mmol) and 50% fuming sulfuric acid (17 mL) in an ice−water bath. After 6 h, the mixture was poured onto ice (108 g). The precipitate was filtered and washed with water to give a colorless solid, which was heated in water, stirred for 2 h at ambient temperature, and filtered to give **26** (10.83 g, 78.6%) as colorless crystals: mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 2.62 (3H, s), 7.52 (1H, d, J = 7.5 Hz), 8.05 (1H, d, J = 8 Hz), 8.09 (1H, d, J = 8 Hz), 8.39 (1H, s), 9.19 (1H, s); MS (FAB) m/z 224 (M + 1).

5-Hydroxy-3-methylisoquinoline (27). A mixture of potassium hydroxide (16.5 g) and sodium hydroxide (16.5 g) was fused at 210 °C, and **26** (10.5 g, 47.0 mmol) was added to the stirred melt portionwise. After 10 min, the reaction mixture was heated at 240 °C for 30 min. The cooled mixture was poured onto ice water (100 mL) and AcOH (35 mL) added therein. The precipitate was filtered, taken up in 2 N HCl, and treated with Celite. The filtrate was neutralized with aqueous ammonia until just alkaline. The precipitate was filtered and crystallized from EtOH to give **27** (1.65 g, 22.0%) as colorless crystals: mp >250 °C; ^{1}H NMR (DMSO-d_6) δ 2.62 (3H, s), 7.04 (1H, d, J = 8 Hz), 7.38 (1H, t, J = 8 Hz), 7.48 (1H, d, J = 8 Hz), 7.77 (1H, s), 9.11 (1H, s). Anal. ($C_{10}H_9NO$) C, H, N.

8-Methoxy-2-methyl-3*H*-quinazolin-4-one (29). To a solution of **28** (10.3 g, 61.6 mmol), 4-(dimethylamino)pyridine (0.65 g), and triethylamine (34.3 mL, 246 mmol) in dry DMF (60 mL) was added dropwise acetyl chloride (10.5 mL, 148 mmol) at 3−15 °C for 20 min in an ice−water bath under nitrogen. The reaction mixture was then heated at 90 °C for 3 h and ammonium carbonate (17.7 g, 185 mmol) added portionwise over 10 min, and the mixture was stirred at the same temperature for 1 h. After cooling, the mixture was poured onto water (300 mL), and the precipitate was filtered and washed with water followed by crystallization from MeCN to give **29** (9.24 g, 78.9%) as colorless crystals: mp 262−263 °C; ^{1}H NMR (DMSO-d_6) δ 2.32 (3H, s), 3.88 (3H, s), 7.30 (1H, d, J = 7.5 Hz), 7.38 (1H, t, J = 7.5 Hz), 7.61 (1H, d, J = 7.5 Hz); MS (FAB) m/z 191 (M + 1). Anal. ($C_{10}H_{10}N_2O_2$) C, H, N.

4-Chloro-8-methoxy-2-methylquinazoline (30). A suspension of **29** (7.50 g, 39.4 mmol) and phosphorus oxychloride (30 mL, 322 mmol) in *N*,*N*-dimethylaniline (10 mL, 78.9 mmol) was heated at 60 °C for 2 h. The reaction mixture was evaporated in vacuo, and the residue was neutralized with saturated aqueous sodium bicarbonate and extracted with CH_2Cl_2 twice. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel chromatography (CH_2Cl_2−AcOEt, 10:1) followed by crystallization from hexane to give **30** (5.32 g, 64.7%) as pale-yellow crystals: mp 101−102 °C; ^{1}H NMR ($CDCl_3$) δ 2.91 (3H, s), 4.09 (3H, s), 7.27 (1H, d, J = 7.5 Hz), 7.58 (1H, t, J = 7.5 Hz), 7.80 (1H, d, J = 7.5 Hz). Anal. ($C_{10}H_9ClN_2O$) C, H, N.

8-Methoxy-2-methylquinazoline (31). A mixture of **30** (4.34 g, 20.8 mmol), 10% palladium on carbon (434 mg), and triethylamine (3.15 g, 31.2 mmol) in AcOEt (43 mL) was stirred at ambient temperature under hydrogen for 3 h. The reaction mixture was filtered through Celite, and the filtrate was washed with saturated aqueous sodium bicarbonate, water,

and brine, dried, and evaporated in vacuo. The residue was crystallized from hexane to give **31** (2.80 g, 77.4%) as colorless crystals: mp 131−132 °C; ^{1}H NMR ($CDCl_3$) δ 2.95 (3H, s), 4.09 (3H, s), 7.21 (1H, d, J = 7.5 Hz), 7.48 (1H, d, J = 7.5 Hz), 7.52 (1H, t, J = 7.5 Hz), 9.30 (1H, s); MS (FAB) m/z 175 (M + 1). Anal. ($C_{10}H_{10}N_2O$) C, H, N.

Compound **37** was prepared using a similar procedure to that used for **31**.

8-Hydroxy-2-methylquinoxaline (34a) and 8-Hydroxy-3-methylquinoxaline (34b). To a suspension of **33** (2.93 g, 23.6 mmol) in 2 M aqueous AcOH solution (47 mL) and 4 M aqueous sodium acetate solution (29 mL) was added 40% aqueous pyruvic aldehyde solution (3.79 mL, 24.8 mmol) at 60 °C. The reaction mixture was then stirred at 60 °C for 40 min. After cooling, the mixture was adjusted to pH 8 with saturated aqueous sodium bicarbonate and extracted with CH_2Cl_2 twice. The organic layer was washed with saturated aqueous sodium bicarbonate, water, and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel chromatography eluting with hexane−AcOEt (5:1) followed by crystallization from hexane to give **34a** (1.27 g, 33.5%) as pale-yellow crystals. Further elution of the column and eluting with hexane−AcOEt (3:1) followed by crystallization from isopropyl ether gave **34b** (1.30 g, 34.3%) as pale-yellow crystals. The structural assignment of **34a** was based on X-ray crystallography. **34a**: mp 83−84 °C; ^{1}H NMR ($CDCl_3$) δ 2.78 (3H, s), 7.21 (1H, m), 7.60 (2H, d, J = 5 Hz), 7.84 (1H, s), 8.79 (1H, s); MS (FAB) m/z 161 (M + 1). Anal. ($C_9H_8N_2O$) C, H, N. **34b**: mp 104−105 °C; ^{1}H NMR ($CDCl_3$) δ 2.80 (3H, s), 7.18 (1H, d, J = 7.5 Hz), 7.55 (1H, d, J = 7.5 Hz), 7.67 (1H, t, J = 7.5 Hz),7.79 (1H, s), 8.60 (1H, s); MS (FAB) m/z 161 (M + 1). Anal. ($C_9H_8N_2O$) C, H, N.

Ethyl *β*-[(3-Methoxyphenyl)amino]crotonate (35). A solution of *m*-anisidine (30.0 g, 0.244 mol), ethyl acetoacetate (31.7 g, 0.244 mol), and AcOH (1 mL) in toluene (60 mL) was refluxed for 8 h removing water with a Dean−Stark apparatus. After cooling, the reaction mixture was concentrated in vacuo. The residue was purified by flash silica gel chromatography (hexane−AcOEt, 20:1) to give **35** (34.7 g, 60.4%) as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.29 (3H, t, J = 7.5 Hz), 2.02 (3H, s), 3.79 (3H, s), 4.15 (2H, q, J = 7.5 Hz), 4.69 (1H, s), 6.61 (1H, d, J = 2 Hz), 6.65−6.73 (2H, m), 7.21 (1H, t, J = 7.5 Hz). Anal. ($C_{13}H_{17}NO_3$) C, H, N.

4-Chloro-5-methoxy-2-methylquinoline (36a) and 4-Chloro-7-methoxy-2-methylquinoline (36b). To Dowtherm was added dropwise **35** (34.6 g, 0.147 mmol) at 225−230 °C over 30 min. After 30 min of stirring at 230 °C, the reaction mixture was cooled. The mixture was diluted with hexane (70 mL) and the precipitate collected by vacuum filtration. The solid was washed with AcOEt to give a ca. 1:4 mixture of 5- and 7-methoxy-2-methyl-4(1*H*)-quinolones (20.6 g, 74.1%) as pale-gray crystals. Using a similar procedure to that used for **30**, this mixture was then converted to the 4-chloroquinolines with phosphorus oxychloride. The separation of isomers was accomplished by silica gel column chromatography eluting with hexane−AcOEt (10:1) to give **36a** (4.50 g, 18.8%) as pale-yellow crystals after recrystallization from hexane and **36b** (16.1 g, 67.4%) as pale-yellow crystals after recrystallization from AcOEt. **36a**: mp 96−97 °C; ^{1}H NMR ($CDCl_3$) δ 2.65 (3H, s), 3.96 (3H, s), 6.89 (1H, dd, J = 8, 1 Hz), 7.28 (1H, d, J = 8 Hz), 7.53−7.65 (2H, m). Anal. ($C_{11}H_{10}ClNO$) C, H, N. **36b**: mp 89−91 °C; ^{1}H NMR ($CDCl_3$) δ 2.69 (3H, s), 3.93 (3H, s), 7.18−7.29 (2H, m), 7.34 (1H, dd, J = 8, 1 Hz), 8.05 (1H, d, J = 8 Hz). Anal. ($C_{11}H_{10}ClNO$) C, H, N.

1-[(*tert*-Butyldiphenylsiloxy)methyl]-2,6-dichloro-3-nitrobenzene (43a). To a solution of **42a** (5.00 g, 22.5 mmol) and imidazole (1.69 g, 24.8 mmol) in DMF (25 mL) was added dropwise *tert*-butyldimethylchlorosilane (6.37 g, 23.1 mmol) at ambient temperature. After 3 h, the mixture was poured into water and extracted with AcOEt. The organic layer was washed with water (2×) and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel chromatography (hexane−AcOEt, 10:1) followed by crystallization from MeOH to give **43a** (9.31 g, 81.5%) as colorless crystals: mp 82−84 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (9H, s), 4.96 (2H, s), 7.27−7.51 (7H, m), 7.58−7.81 (5H, m). Anal. ($C_{23}H_{23}Cl_2NO_3Si$) C, H, N.

Compound **43b** was prepared using a similar procedure to that used for **43a**.

1-[(*tert*-Butyldiphenylsiloxy)methyl]-2,6-dichloro-3-[(*N*-phthalimidoacetyl)amino]benzene (45a). To a solution of **44a** (3.00 g, 6.97 mmol) and triethylamine (1.46 mL, 10.5 mmol) in dry CH_2Cl_2 (30 mL) was added *N*-phthalimidoacetyl chloride (1.87 g, 8.36 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 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 (hexane−AcOEt, 5:1) followed by crystallization from isopropyl ether to give **45a** (3.84 g, 89.3%) as colorless crystals: mp 198−199 °C; ^{1}H NMR ($CDCl_3$) δ 1.04 (9H, s), 4.57 (2H, s), 4.90 (2H, s), 7.25−7.49 (7H, m), 7.70−7.82 (6H, m), 7.90−7.97 (2H, m), 8.00 (1H, br s), 8.26 (1H, d, J = 8 Hz). Anal. ($C_{33}H_{30}Cl_2N_2O_4Si$) C, H, N.

Compounds **45b** and **94** were prepared using a similar procedure to that used for **45a**.

3-(*N*-Aminoacetyl-*N*-methylamino)-1-[(*tert*-butyldiphenylsiloxy)methyl]-2,6-dichlorobenzene (47a). To a suspension of **46a** (3.60 g, 5.70 mmol) in EtOH (36 mL) was added hydrazine monohydrate (571 mg, 11.4 mmol) at ambient temperature, and the mixture was refluxed for 1 h. After the reaction mixture was cooled, the precipitates formed were filtered off. The filtrate was evaporated in vacuo, CH_2Cl_2 (20 mL) was added to the residue, and precipitates were filtered off. The filtrate was again evaporated in vacuo and the residue purified by flash silica gel column chromatography (CH_2Cl_2−MeOH, 20:1) to give **47a** (2.65 g, 92.6%) as a pale-yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 1.05 (9H, s), 2.94 (1H, d, J = 17 Hz), 3.09 (1H, d, J = 17 Hz), 3.20 (3H, s), 4.93 (2H, s), 7.18 (1H, d, J = 8 Hz), 7.35−7.49 (7H, m), 7.69−7.77 (4H, m). Anal. ($C_{26}H_{30}Cl_2N_2O_2Si$) C, H, N.

Compounds **47b** and **69a**,**b**−**71a**,**b** were prepared using a similar procedure to that used for **47a**.

2,6-Dichloro-1-hydroxymethyl-3-[*N*-methyl-*N*-[(*E*)-4-(*N*-methylcarbamoyl)cinnamamidoacetyl]amino]-benzene (49a). To a suspension of **48a** (1.76 g, 2.56 mmol) in THF (14 mL) was added 1 M tetrabutylammonium fluoride in THF (3.84 mL) at ambient temperature. After 1 h, the mixture was partitioned between $CHCl_3$ and water. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was crystallized from MeCN to give **49a** (933 mg, 81.0%) as colorless crystals: mp 208−211 °C; ^{1}H NMR (DMSO-d_6) δ 2.79 (3H, d, J = 5 Hz), 3.11 (3H, s), 3.47 (1H, dd, J = 17, 4 Hz), 3.77 (1H, dd, J = 17, 5 Hz), 4.74 (1H, d, J = 5 Hz), 5.34 (1H, t, J = 5 Hz), 6.87 (1H, d, J = 15 Hz), 7.40 (1H, d, J = 15 Hz), 7.59−7.68 (4H, m), 7.85 (2H, d, J = 8 Hz), 8.29 (1H, t, J = 5 Hz), 8.48 (1H, d, J = 5 Hz); MS (FAB) m/z 648 (M + 1). Anal. ($C_{21}H_{21}Cl_2N_3O_4$) C, H, N.

Compounds **49b**,**c** and **65a**,**b** were prepared using a similar procedure to that used for **49a**.

1-Bromomethyl-2,6-dichloro-3-[*N*-methyl-*N*-[(*E*)-4-(*N*-methylcarbamoyl)cinnamamidoacetyl]amino]benzene (50a). To a mixture of **49a** (8.10 g, 18.0 mmol) in CH_2Cl_2 (81 mL) was added triphenylphosphine (5.66 g, 21.6 mmol) and carbon tetrabromide (8.95 g, 27.0 mmol) in an ice−water bath. After 15 min, the reaction mixture was stirred at ambient temperature for 3 h. To the mixture were added triphenylphosphine (1.42 g, 5.40 mmol) and carbon tetrabromide (2.39 g, 7.20 mmol), and stirring was continued for a further 2 h. The reaction mixture was washed with saturated sodium hydrogen carbonate, water, and brine, and the organic layer was dried and evaporated in vacuo. The resulting residue was purified by flash silica gel column chromatography (CH_2Cl_2−MeOH, 20:1) followed by crystallization from AcOEt to give **50a** (6.40 g, 69.3%) as pale-yellow crystals: mp 212−215 °C; ^{1}H NMR ($CDCl_3$) 3.02 (3H, d, J = 5 Hz), 3.27 (3H, s), 3.62

(1H, dd, J = 17, 4 Hz), 3.92 (1H, dd, J = 17, 5 Hz), 4.78 (1.2H, s), 4.90 (0.8H, s), 6.15 (1H, br d, J = 5 Hz), 6.51 (1H, d, J = 15 Hz), 6.67 (1H, br t, J = 5 Hz), 7.29 (1H, overlapped with H_2O), 7.45−7.62 (4H, m), 7.76 (2H, d, J = 8 Hz). Anal. ($C_{21}H_{20}BrCl_2N_3O_3$) C, H, N.

1-Acetoxymethyl-2,6-dimethylbenzene (40). A mixture of **39** (13.6 g, 100 mmol) and acetic anhydride (11.3 mL, 120 mmol) was stirred at 70 °C for 5 h during which time to the mixture was added 4-(dimethylamino)pyridine (10 mg). After cooling, the reaction mixture was evaporated in vacuo. The residue was dissolved in AcOEt and washed with water twice. The organic layer was dried and evaporated in vacuo. The residue was purified by flash silica gel chromatography (hexane−AcOEt, 10:1) to give **40** (17.5 g, 98.2%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.07 (3H, s), 2.38 (6H, s), 5.19 (2H, s), 7.05 (2H, d, J = 8 Hz), 7.15 (1H, t, J = 8 Hz). Anal. ($C_{11}H_{14}O_2$) C, H, N.

1-Acetoxymethyl-2,6-dimethyl-3-nitrobenzene (41). To a solution of acetic anhydride (34 mL) and AcOH (17 mL) was added copper(II) nitrate trihydrate (16.3 g, 67.3 mmol) in an ice−water bath. To this stirred mixture was added dropwise a solution of **40** (10.0 g, 56.1 mmol) in acetic anhydride (10 mL) and AcOH (5 mL) over 30 min. The reaction mixture was stirred for 30 min in an ice−water bath and 30 min at ambient temperature. This mixture was poured onto ice (250 g) and extracted with AcOEt twice. The organic layer was washed with water (3×) and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel chromatography (hexane−AcOEt, 5:1) to give **41** (9.59 g, 94.3%) as a pale-yellow oil: ^{1}H NMR ($CDCl_3$) δ 2.08 (3H, s), 2.47 (3H, s), 2.50 (3H, s), 5.22 (2H, s), 7.18 (1H, d, J = 8 Hz), 7.69 (1H, d, J = 8 Hz). Anal. ($C_{11}H_{13}NO_4$) C, H, N.

1-Chloromethyl-2,6-dimethyl-3-[*N*-methyl-*N*-[(*E*)-4-(*N*-methylcarbamoyl)cinnamamidoacetyl]amino]benzene (50b). To a solution of **49b** (2.00 g, 4.89 mmol) and triethylamine (990 mg, 9.78 mmol) in dry DMF (100 mL) was added methanesulfonyl chloride (784 mg, 6.85 mmol) in an ice−water bath under nitrogen. After 30 min, the reaction mixture was stirred at ambient temperature for 14 h. The mixture was partitioned between CH_2Cl_2 and water. The organic layer was washed with water (4×) and brine, dried, and evaporated in vacuo. The residue was crystallized from AcOEt to give **50b** (1.89 g, 90.4%) as colorless crystals: mp 232−233 °C; ^{1}H NMR ($CDCl_3$) δ 2.29 (3H, s), 2.46 (3H, s), 3.03 (3H, d, J = 5 Hz), 3.24 (3H, s), 3.59 (1H, dd, J = 17, 5 Hz), 3.82 (1H, dd, J = 17, 4 Hz), 4.67 (2H, s), 6.20 (1H, m), 6.50 (1H, d, J = 15 Hz), 6.70 (1H, d, J = 5 Hz), 7.04 (1H, d, J = 9 Hz), 7.14 (1H, d, J = 9 Hz), 7.50−7.60 (3H, m), 7.75 (2H, d, J = 9 Hz). Anal. ($C_{23}H_{26}ClN_3O_3$) C, H, N.

Compound **50c** was prepared using a similar procedure to that used for **50b**.

Method A. 8-[[2,6-Dichloro-3-[*N*-methyl-*N*-[(*E*)-4-(*N*-methylcarbamoyl)cinnamamidoacetyl]amino]benzyl]-oxy]-2-methylquinoline (62a). To a solution of 8-hydroxy-2-methylquinoline (100 mg, 0.628 mmol) in dry DMF (2 mL) was added 60% sodium hydride in oil (27 mg, 0.659 mmol) in an ice−water bath under nitrogen. After 30 min, **50a** (256 mg, 0.598 mmol) was added therein, followed by stirring at ambient temperature for 2 h. The mixture was poured into water and extracted with $CHCl_3$ twice. The extracts were washed with water (3×) and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel column chromatography (CH_2Cl_2−MeOH, 30:1) followed by crystallization from AcOEt to give a colorless solid (315 mg, 88.7%). The solid was recrystallized from MeOH to afford **62a** (280 mg, 78.9%) as colorless crystals: mp 232−234 °C; ^{1}H NMR ($CDCl_3$) δ 2.71 (3H, s), 3.00 (3H, d, J = 5 Hz), 3.26 (3H, s), 3.64 (1H, dd, J = 17, 4 Hz), 3.94 (1H, dd, J = 17, 5 Hz), 5.65 (2H, s), 6.41 (1H, br d, J = 5 Hz), 6.53 (1H, d, J = 15 Hz), 6.73 (1H, br s), 7.30 (2H, d, J = 8 Hz), 7.37−7.61 (7H, m), 7.76 (2H, d, J = 8 Hz), 8.03 (1H, d, J = 8 Hz). Anal. ($C_{31}H_{28}Cl_2N_4O_4$) C, H, N.

Compounds **14a**,**b**, **20d**, **23**, **46a**,**b**, **51**, **52a**−**j**,**l**,**m**, **53**, **55**, **56**, **58**−**63a**,**b**, and **69a**,**b**−**71a**,**b** were prepared following a procedure similar to method A.

Method B. 8-[[2,6-Dichloro-3-[*N*-methyl-*N*-[(*E*)-4-(*N*-methylcarbamoyl)cinnamamidoacetyl]amino]benzyl]-oxy]-2-methylquinoline Hydrochloride (54a). To a suspension of **62a** (500 mg, 0.845 mmol) in MeOH (5 mL) was added 10% hydrogen chloride in MeOH (2 mL) at ambient temperature. After 10 min, the solution was evaporated in vacuo. The residue was crystallized from MeCN to give **54a** (498 mg, 93.8%) as colorless crystals: mp 160−165 °C; ^{1}H NMR ($CDCl_3$−CD_3OD) δ 2.98 (3H, s), 3.10 (3H, s), 3.31 (3H, s), 3.89 (1H, d, J = 17 Hz), 4.05 (1H, d, J = 17 Hz), 5.59 (1H, d, J = 10 Hz), 5.75 (1H, d, J = 10 Hz), 6.65 (1H, d, J = 16 Hz), 7.37−7.73 (6H, m), 7.73−8.00 (5H, m), 8.92 (1H, d, J = 8 Hz); MS (FAB) m/z 591; IR (KBr) 3491, 3418, 3238, 3038, 1673, 1660, 1635, 1612, 1596, 1541. Anal. ($C_{31}H_{28}Cl_2N_4O_4$·HCl·H_2O) C, H, N.

Compounds **54b**−**d**, **57a**, **64c**, **76a**, **81a**,**b**, **86**, **88a**,**c**, **90a**−**d**, **93b**,**c**, **95**, and **98** were prepared following a procedure similar to method B.

Method C. 8-[[2,6-Dichloro-3-[*N*-methyl-*N*-[(*E*)-3-[6-(*N*-methylcarbamoyl)pyridin-3-yl]acryloylglycyl]amino]-benzyl]oxy]-2-methylquinoline (62c). To a solution of **83a** (100 mg, 0.173 mmol) in dry DMF (1 mL) were added methylamine hydrochloride (14 mg, 0.207 mmol), WSCD (38 mg, 0.242 mmol), and HOBt (37 mg, 0.277 mmol) at ambient temperature. After 3 h, this mixture was partitioned between AcOEt and saturated aqueous sodium bicarbonate solution. The organic layer was washed with water (3×) 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 **62c** (65 mg, 63.8%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 2.73 (3H, s), 3.04 (3H, d, J = 5 Hz), 3.28 (3H, s), 3.70 (1H, dd, J = 18, 4 Hz), 3.95 (1H, dd, J = 18, 5 Hz), 5.64 (2H, s), 6.64 (1H, d, J = 16 Hz), 6.76 (1H, br s), 7.21−7.37 (3H, m), 7.37−7.54 (3H, m), 7.60 (1H, d, J = 16 Hz), 7.88−8.09 (3H, m), 8.19 (1H, d, J = 8 Hz), 8.62 (1H, br d, J = 5 Hz); MS (FAB) m/z 592 (M + 1). Anal. ($C_{30}H_{27}Cl_2N_5O_4$) C, H, N.

Compounds **62d**, **63c**, and **84** were prepared following a procedure similar to method C.

8-[[2,6-Dichloro-3-[*N*-methyl-*N*-(*N*-phthalimidoacetyl)-amino]benzyl]oxy]-2-methylquinoline (68a). To a solution of **65a** (4.33 g, 11.0 mmol) and triethylamine (1.67 g, 16.5 mmol) in dry CH_2Cl_2 (43 mL) was added dropwise methanesulfonyl chloride (1.39 g, 12.1 mmol) in an ice−water bath under nitrogen. After 30 min, the reaction mixture was washed with water, saturated aqueous sodium bicarbonate, and brine. The organic layer was dried and evaporated in vacuo to give 5.18 g of a pale-yellow oil. Following a procedure similar to method A, the title compound was obtained in 91.6% yield from 8-hydroxy-2-methylquinoline and the preceding oil as colorless crystals after crystallization from MeOH: mp 211−213 °C; ^{1}H NMR ($CDCl_3$) δ 2.72 (3H, s), 3.23 (3H, s), 4.08 (2H, s), 5.66 (1H, d, J = 10 Hz), 5.72 (1H, d, J = 10 Hz), 7.20−7.49 (4H, m), 7.48 (1H, d, J = 8 Hz), 7.55 (1H, d, J = 8 Hz), 7.65−7.77 (2H, m), 7.80−7.93 (2H, m), 8.00 (1H, d, J = 8 Hz). Anal. ($C_{28}H_{21}Cl_2N_3O_4$) C, H, N.

Compounds **66**, **67a**,**b**, and **68b** were prepared using a similar procedure to that used for **68a**.

Method D. 8-[[3-[*N*-(*E*)-Cinnamamidoacetyl-*N*-methyl-amino]-2,6-dichlorobenzyl]oxy]-2-methylquinoline (80a). To a solution of **71a** (100 mg, 0.247 mmol) and triethylamine (38 mg, 0.375 mmol) in dry CH_2Cl_2 (2 mL) was added (*E*)-cinnamoyl chloride (45 mg, 0.270 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 for 1 h. The reaction mixture was washed with water, saturated aqueous sodium bicarbonate, and brine, dried, and evaporated in vacuo. The residue was then purified by flash silica gel column chromatography (hexane−AcOEt, 1:4) to give **80a** (118 mg, 89.4%) as a colorless amorphous solid: ^{1}H NMR ($CDCl_3$) δ 2.73 (3H, s), 3.26 (3H, s), 3.65 (1H, dd, J = 17, 4 Hz), 3.85 (1H, dd, J = 17, 5 Hz), 5.65 (2H, s), 6.48 (1H, d, J = 16 Hz), 6.65 (1H, br t, J = 5 Hz), 7.19−7.53

(11H, m), 7.57 (1H, d, J = 16 Hz), 8.02 (1H, d, J = 8 Hz). Anal. ($C_{29}H_{25}Cl_2N_3O_3$) C, H, N.

Compounds **9j**, **13b**−**d**,**f**, and **59a**,**b** were prepared following a procedure similar to method D.

8-[[3-[*N*-(*E*)-4-Carboxycinnamamidoacetyl-*N*-methylamino]-2,6-dichlorobenzyl]oxy]-2-methylquinoline (83a). A solution of **82a** (1.00 g, 1.69 mmol) in MeOH (17 mL) and 1,4-dioxane (17 mL) containing 1 N NaOH (3.4 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 **83a** (1.42 g, 83.9%) as colorless crystals: mp 239−241 °C; ^{1}H NMR (DMSO-d_6) δ 2.61 (3H, s), 3.15 (3H, s), 3.51 (1H, dd, J = 18, 4 Hz), 3.81 (1H, dd, J =18, 4 Hz), 5.48 (1H, d, J = 10 Hz), 5.54 (1H, d, J = 10 Hz), 6.90 (1H, d, J = 16 Hz), 7.32−7.60 (5H, m), 7.64−7.75 (2H, m), 7.75−7.85 (2H, m), 7.96 (2H, d, J = 8 Hz), 8.21 (1H, d, J = 8 Hz), 8.40 (1H, m); MS (FAB) m/z 578 (M + 1). Anal. ($C_{30}H_{25}Cl_2N_3O_5$) C, H, N.

Compounds **12**, **42b**, **79**, **83b**−**d**, and **106** were prepared using a similar procedure to that used for **83a**.

Method E. 8-[[3-[*N*-[(*E*)-3-(6-Acetamidopyridin-3-yl)acryloylglycyl]-*N*-methylamino]-2,6-dichlorobenzyl]oxy]-2-methylquinoline (87b). To a solution of **71a** (500 mg, 1.24 mmol), (*E*)-3-(6-acetamidopyridin-3-yl)acrylic acid[23] (281 mg, 1.36 mmol), and 1-hydroxybenzotriazole (HOBt; 217 mg, 1.61 mmol) in dry DMF (5 mL) was added 1-ethoxy-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (WSCD·HCl; 285 mg, 1.48 mmol) in an ice−water bath under nitrogen. After 30 min, the reaction mixture was stirred at ambient temperature for 2 h. The mixture was partitioned between $CHCl_3$ and saturated aqueous sodium bicarbonate solution. The organic layer was washed with water (3×) and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel column chromatography ($CHCl_3$−MeOH, 30:1) followed by crystallization from MeCN to give **87b** (576 mg, 78.4%) as colorless crystals: mp 146−150 °C; ^{1}H NMR ($CDCl_3$) δ 2.22 (3H, s), 2.74 (3H, s), 3.27 (3H, s), 3.67 (1H, dd, J = 17, 4 Hz), 3.96 (1H, dd, J = 17, 5 Hz), 5.62 (1H, d, J = 11 Hz), 5.67 (1H, d, J = 11 Hz), 6.46 (1H, d, J = 15 Hz), 6.73 (1H, br t, J = 4 Hz), 7.21−7.33 (3H, m), 7.38−7.51 (3H, m), 7.52 (1H, d, J = 15 Hz), 7.82 (1H, dd, J = 8, 1 Hz), 8.03 (1H, d, J = 8 Hz), 8.13−8.25 (2H, m), 8.33 (1H, d, J = 1 Hz); MS (ESI) m/z 592 (M + 1); IR (KBr) 3587, 3501, 3381, 2962, 1682, 1661, 1615, 1582, 1565, 1535, 1509. Anal. ($C_{30}H_{27}Cl_2N_5O_4$·$2H_2O$) C, H, N.

Compounds **48a**−**c**, **72**−**75a**,**b**, **77a**,**b**, **78**, **80b**, **82a**−**d**, **85**, **87a**,**c**, **89a**−**d**, **91**, **92a**−**c**, **94**, and **96** were prepared following a procedure similar to method E.

Methyl 6-[(*E*)-2-(4-Pyridinyl)ethenyl]pyridine-3-carboxylate (102). A mixture of **100** (10.0 g, 66.2 mmol) and **101** (10.6 g, 99.2 mmol) in acetic anhydride (60 mL) and AcOH (60 mL) was stirred at 120 °C for 5 days under nitrogen. The mixture was evaporated in vacuo, and the residue was dissolved in $CHCl_3$, washed with saturated aqueous sodium bicarbonate (2×) and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel column chromatography (hexane−AcOEt, 1:3) followed by crystallization from isopropyl ether to give **102** (3.92 g, 24.7%) as yellow crystals: mp 126−127 °C; ^{1}H NMR ($CDCl_3$) δ 3.96 (3H, s), 7.36 (1H, d, J = 16 Hz), 7.40−7.51 (3H, m), 7.71 (1H, d, J = 16 Hz), 8.30 (1H, dd, J = 8, 1 Hz), 8.63 (2H, d, J = 7 Hz), 9.21 (1H, br s). Anal. ($C_{14}H_{12}N_2O_2$) C, H, N.

3-Formyl-6-[(*E*)-2-(4-pyridinyl)ethenyl]pyridine-3-carboxylate (104). To a mixture of **103** (246 mg, 1.16 mmol) and triethylamine (586 mg, 5.80 mmol) in dry DMSO (3 mL) and dry CH_2Cl_2 (2 mL) was added sulfur trioxide pyridine complex (554 mg, 3.48 mmol) in portions at ambient temperature under nitrogen. After 1 h, the reaction mixture was partitioned between AcOEt and water. The organic layer 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 (hexane−AcOEt, 1:4) followed by crystallization from isopropyl ether to give **104** (191 mg, 78.4%) as pale-yellow crystals: mp 132−136 °C; ^{1}H NMR ($CDCl_3$) δ 7.40 (1H, d, J = 16 Hz), 7.47 (2H, d, J = 6 Hz), 7.56 (1H, d, J = 8 Hz), 7.78 (1H, d, J = 16 Hz), 8.19 (1H, dd, J = 8, 2 Hz), 8.65 (2H, d, J = 6 Hz), 9.07 (1H, d, J = 2 Hz), 10.12 (1H, s). Anal. ($C_{13}H_{10}N_2O$) C, H, N.

Methyl 3-[6-[(*E*)-2-(4-Pyridinyl)ethenyl]pyridin-3-yl]-acrylate (105). To a solution of **104** (191 g, 0.908 mmol) in dry THF (2 mL) was added methyl (triphenylphosphoranylidene)acetate (364 mg, 1.09 mmol) at ambient temperature under nitrogen. After 2 h, the reaction mixture was partitioned between AcOEt and water. The organic layer was washed with water and brine, dried, and evaporated in vacuo. The residue was purified by flash silica gel column chromatography (CH_2Cl_2−MeOH, 30:1) followed by crystallization from isopropyl ether to give **105** (183 mg, 89.2%) as pale-yellow crystals: mp 143−145 °C; ^{1}H NMR ($CDCl_3$) δ 3.83 (3H, s), 6.53 (1H, d, J = 16 Hz), 7.34 (1H, d, J = 16 Hz), 7.40−7.47 (3H, m), 7.64 (1H, d, J = 16 Hz), 7.70 (1H, d, J = 16 Hz), 7.87 (1H, d, J = 8 Hz), 8.63 (2H, d, J = 7 Hz), 8.75 (1H, d, J = 2 Hz); MS (ESI) m/z 266.8 (M + 1). Anal. ($C_{16}H_{14}N_2O_2$) C, H, N.

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 (1000*g*, 20 min, 4 °C), and the supernatant was centrifuged (100000*g*, 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.

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. 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 deter-

mined 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.

Recombinant Human B_2 Receptors Expressed in CHO Cells. CHO (dhfr$^-$) cells, which were transferred with and stably expressed human B_2 receptors, have been described previously.[22b] 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 [^{3}H]BK and test compounds for 2 h at 4 °C in 0.25 mL of binding buffer III (20 mM HEPES, 125 mM *N*-methyl-D-glucamine, 5.0 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.05 mM bacitracin, 5 μM enalaprilat, and 0.1% BSA, pH 7.2). All 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. 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% SDS containing 0.05 N NaOH and quantitating in a liquid scintillation counter.

BK-Induced Bronchoconstriction in Guinea Pigs. Male Hartley guinea pigs weighing 470−750 g (from Charles River Japan, Inc.) were fasted overnight and anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg), 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 = $(\Delta PIP_{\text{after drug}}/\Delta PIP_{\text{before drug}}) \times 100$. 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 = $(1 - \%\ \text{response}_{\text{drug}}/\%\ \text{response}_{\text{mean value of vehicle}}) \times 100$.

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.

Acknowledgment. We are grateful to Dr. D. Barrett (Medicinal Chemistry Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka) and Dr. N. Maeda (Department of Pharmacology, Fujisawa Pharmaceutical Co., Ltd., Tsukuba) for their valuable suggestions.

Supporting Information Available: X-ray crystallographic data of **34a** and physical data of **12**−**14a**,**b**, **15b**, **16b**−**e**, **17b**−**e**, **18b**−**e**, **20b**,**d**,**e**, **21a**−**d**,**f**, **22**, **23**, **32**, **37**, **38**, **42b**, **43b**, **44a**,**b**, **45b**, **46a**,**b**, **47b**, **48a**−**c**, **49b**,**c**, **50c**, **51**, **52a**−**j**,**l**,**m**, **53**, **54b**−**d**, **55**−**61**, **62b**,**d**−**67a**,**b**, **68b**−**79**, **80b**−**83b**,**c**,**d**, **84**−**87a**,**c**, **88a**,**c**−**93b**,**c**, **94**−**99**, **103**, and **106** (49 pages). Ordering information is given on any current masthead page.

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JM980300F