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From Bradykinin B2 Receptor Antagonists to Orally Active and Selective Bradykinin B1 Receptor Antagonists

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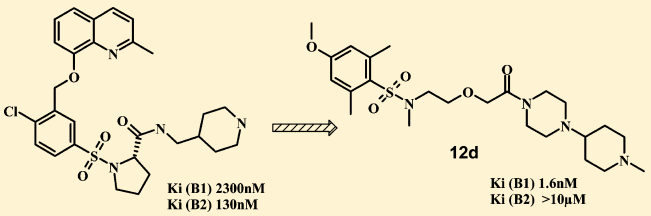
Supporting Information

ABSTRACT: The bradykinin (BK) B1 receptor is an attractive target for the treatment of chronic pain and inflammation. Starting from a dual B1 and B2 antagonist, novel antagonists were designed that display low-nanomolar affinity for human B1 receptor and selectivity over B2. Initially, potent imidazoline derivatives were studied, but these compounds suffered from low bioavailability. This issue could be overcome by the use of less basic amino derivatives leading to orally active compounds.



INTRODUCTION

Kinins are 9−11 amino acid peptides known to be important mediators of pain, inflammation, and cardiovascular homeostasis. They are released in injured tissues from kininogen by activation of plasma or tissue kallikreins.[1] Kinins exert their biological activities via the activation of two subtypes of G-protein-coupled receptors, namely, B1 and B2 receptors.[2,3]

Bradykinin (BK) and Lys0-BK are natural endogenous agonists of BK B2 receptor, while their kininase I hydrolyzed metabolites desArg9-BK and Lys0-desArg9-BK are specific agonists of BK B1 receptors. Whereas B2 receptors are constitutively present in normal tissues, B1 receptors are poorly present in healthy tissues. B1 receptors are highly inducible by tissue injury and treatment by bacterial endotoxin or inflammatory mediators such as cytokines.[4,5]

The contribution of B1 receptor activation in inflammation and pain process is supported by the demonstration that B1 receptor knockout mice have a decreased response to nociceptive and proinflammatory stimuli.[6] The therapeutic interest of B1 receptor blocking is further supported by the pharmacological properties of B1 peptide antagonist desArg9,Leu8-BK.[7,8] A number of experimental studies using B1 receptor antagonists support a major role for B1 receptors in hyperalgesia.[9] Accordingly identification of selective and effective BK B1 receptor antagonists may have potential for the treatment against chronic inflammation and pain.

Our effort to find effective small molecule B1 receptor antagonists was initiated with a high-throughput screen of our in-house compound library. Identified hits belong to our B2 receptor antagonist research program. Only one of them, a benzenesulfonamide derivative (Figure 1), showed functional activity. This compound has 18-fold higher affinity for the B2 receptor than for the B1 receptor (B1 K_i = 2.3 μM and B2 K_i = 0.13 μM).

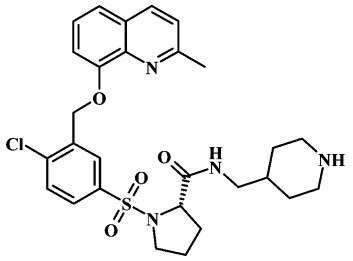


Figure 1. Identified hit.

Molecular modeling and mutagenesis data showed that the quinoline part of this B2 antagonist interacts simultaneously with Tyr295 in transmembrane spanning domain 7 (TM7) and Trp256 (TM6) in the B2 receptor.[10] This sandwich interaction is essential for stabilizing the B2 receptor in its inactive conformation. This essential role of the quinoline was also supported by the dramatic loss of affinity induced by Ala or Phe mutation of Tyr295. Actually, deletion of the quinoline part of the molecule led to **1a** (Figure 2) with the loss of B2 antagonist activity while keeping the B1 activity. Compound **1a** is 20-fold selective for B1 versus B2 receptor with B1 K_i = 2.3 μM and B2 K_i = 43 μM.

The central proline was replaced by various amino acids and dipeptides in order to find new interactions with the receptor. This systematic study led to **1b** (Figure 2) with improved B1 affinity (K_i = 59 nM, pK_B = 6.6). Peptide compounds being potentially prone to peptidase, a peptoid strategy was used. Amino acid side chains were shifted on one amidic nitrogen atom, leading to **1c** (Figure 2) with improved functional activity (K_i = 100 nM, pK_B = 7.5). In order to improve B1 affinity, SAR

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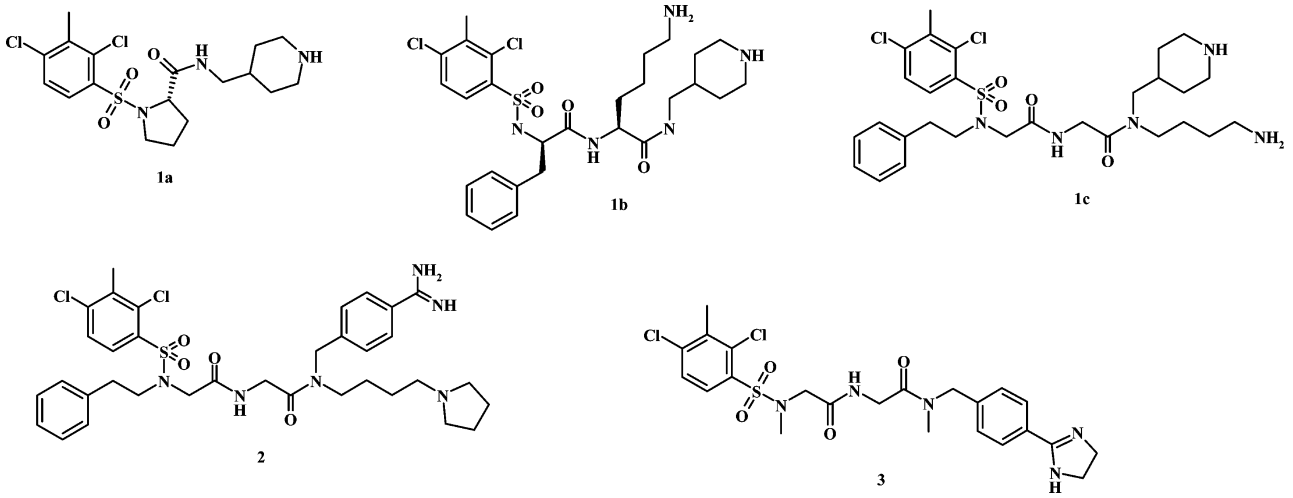
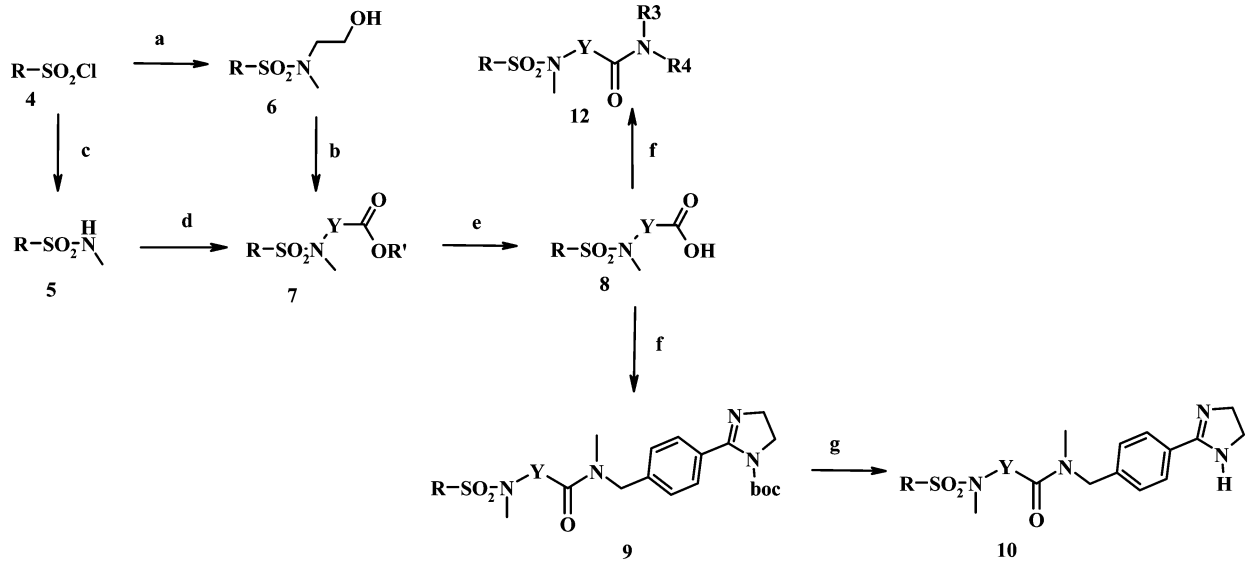


Figure 2. Leads after first SAR round.

Scheme 1. Synthesis of Arylsulfonamide B1 Antagonists: First Route[a]



[a](a) $CH_3NHCH_2CH_2OH$, Et_3N, CH_2Cl_2, rt; (b) $BrCH_2COOtBu$, *n*-Bu_4NCl, 35% NaOH, toluene, rt; (c) $MeNH_2$·HCl, Et_3N, CH_2Cl_2, rt; (d) Br-Y-COOEt, K_2CO_3, DMF, rt; (e) LiOH, THF, H_2O, rt; (f) EDCI, HOAT, Aamine, CH_2Cl_2, rt; (g), TFA, anisole, CH_2Cl_2, rt.

of the terminal diamino-containing part was done. The result of these investigations was the discovery of a highly potent B1 receptor antagonist **2** (Figure 2) (affinity K_i = 1.5 nM and functional activity pK_B = 9.1). Unfortunately, this compound did not appear as a good lead for oral treatment of inflammatory hyperalgesia. It suffered from a high molecular weight (MW = 715), the presence of two basic groups (calculated pK_a of 10.5 and 12), and an important flexibility (number of rotatable bonds, >15). As anticipated, it lacked oral activity. The efforts were thus focused toward reducing both molecular weight and basicity of the compounds while keeping enough affinity for the human B1 receptor. This led to **3** (Figure 2) (pK_B = 7.2 and MW = 540). This paper reports structure−activity relationship (SAR) and absorption, distribution, metabolism, and excretion (ADME) optimization of these compounds that lead to potent selective B1 antagonists exhibiting excellent pharmacokinetic profiles.

RESULTS AND DISCUSSION

Synthesis. The compounds described in this paper, were prepared according to two routes depicted in Schemes 1 and 2. Route 2 was specially designed for SAR of the arylsulfonyl part, introducing this moiety in the latest stages.

In Scheme 1, esters **7** were obtained according to two routes depending on the linker Y. For ether containing linker, compounds **7** were prepared in two steps from commercially or known arylsulfonyl chlorides. The sulfonylation of 2-(methylamino)ethanol, giving **6**, was followed by O-alkylation with *tert*-butyl bromoacetate under phase transfer conditions (*n*-Bu_4NCl, NaOH, toluene). For all other linkers, compounds **7** were also synthesized in two steps: sulfonylation of methylamine to give **5**, followed by N-alkylation with Br-Y-COOR using K_2CO_3 in DMF at room temperature. Esters **7** were saponified with lithium hydroxide to give the corresponding acids **8**. These acids were coupled with various amines (commercially available, synthesized according to Scheme 3 or literature[11]), using 1-ethyl-3-(3-dimethylaminopropyl)-

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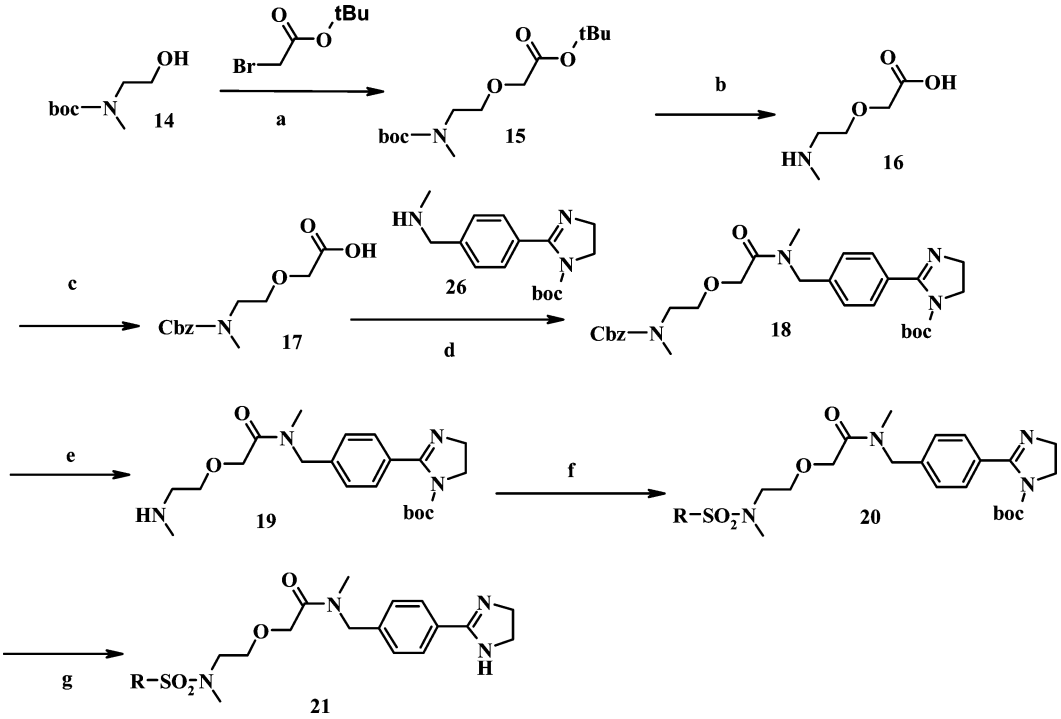
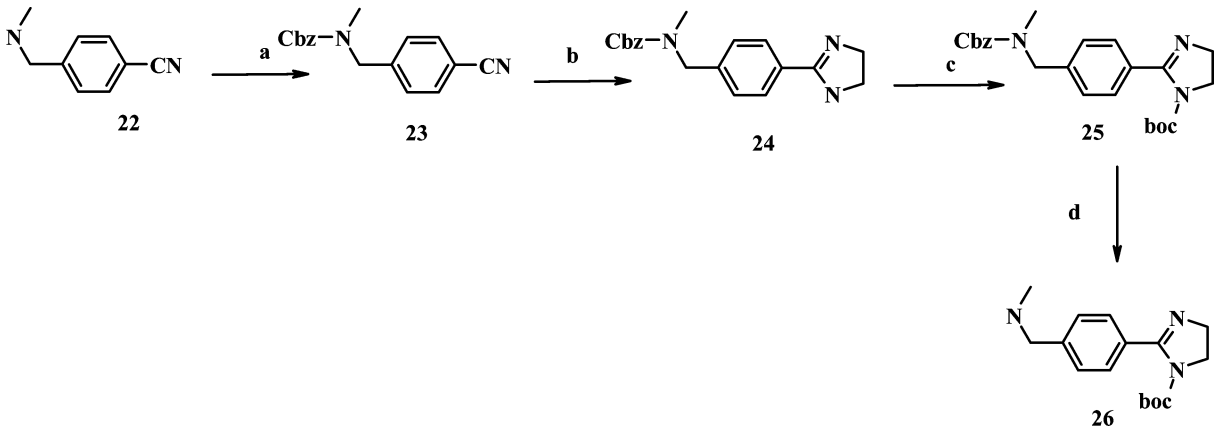
Scheme 2. Synthesis of Arylsulfonamide B1 Antagonists: Second Route[a]



[a](a) $BrCH_2COOtBu$, n-Bu_4NCl, 35% NaOH, toluene, rt; (b), TFA, CH_2Cl_2; (c) CbzCl, Et_3N, CH_2Cl_2, rt; (d) EDCI, HOAT, amine, CH_2Cl_2, rt; (e) H_2, Pd/C 10%, MeOH, rt; (f) RSO_2Cl, Et_3N, CH_2Cl_2, rt; (g) TFA, anisole, CH_2Cl_2.

Scheme 3. Synthesis of Phenylimidazoline Synthons[a]



[a](a) CbzCl, Et_3N, CH_2Cl_2 rt; (b) S, $H_2NCH_2CH_2NH_2$, 100 °C; (c) Boc_2O, DMAP, CH_2Cl_2, rt; (d) H_2, 10% Pd/C, MeOH, rt.

carbodiimide (EDCI) and 1-hydroxy-7-azabenzotriazole (HOAT) in dichloromethane to give either final compounds **12a–r** or intermediates **9**. Final compounds **10** were obtained after Boc protective group cleavage of **9** with trifluoroacetic acid.

In Scheme 2, alkylation of Boc-protected 2-(methylamino)-ethanol **14** with *tert*-butyl bromoacetate gave diprotected amino acid **15**. Cleavage of both protective groups (*tert*-butylcarbamate and *tert*-butyl ester) using TFA followed by protection of the amino residue with benzylcarbamate gave acid **17** (the synthesis of **17** starting from *N*-(benzyloxycarbonyl)-2-amino-ethanol was unsuccessful (unpublished results)). Coupling of acid **17** with amine **26** (described in Scheme 3), using EDCI, HOAT in dichloromethane, was followed by hydrogenolysis of the benzylcarbamate group to give the amine derivative **19**. Compound **19** was sulfonylated with commercially available arylsulfonyl chlorides using triethylamine in dichoromethane, followed by Boc group cleavage with TFA, to give final products **21**.

The 2-phenylimidazoline derivative **26** was prepared as described in Scheme 3. Benzylcarbamate protection of **22** was followed by imidazoline formation using 1,2-diaminoethane and sulfur to give **24**. *tert*-Butylcarbamate protection of the imadzoline group and final cleavage of Cbz group gave the desired synthon **26**.

Biological Activity. K_i values were calculated from concentration curves in competition-binding assays using [^{3}H]des-Arg^{10}-kallidin as a specific ligand for the B_1 receptor with membrane preparations either from human endothelium kidney cells 293 (HEK293) or from monkey kidney fibroblast

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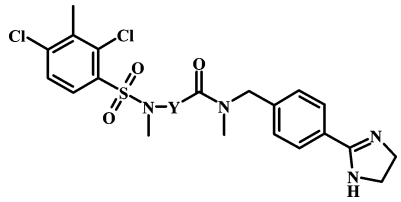
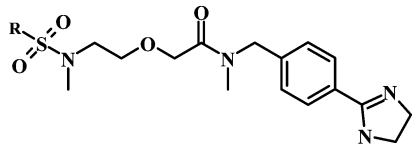
Table 1. Linker SAR: Binding Affinities and Functional Activities of B1 Receptor Antagonists



compd	Y	K_i [a] (nM) (human)	pK_B [b] (human)	pK_B [b] (mouse)	metabolic stability[c] (human)
3	$CH_2CONHCH_2$	78	7.2	6.4	2
10a	$(CH_2)_4$	nd	7.3	6	0
10b	$(CH_2)_3$	65	7.5	5.6	8
10c	$(CH_2)_2$	15	8.5	6.4	5
10d	$CH_2CH_2OCH_2$	2.1	9.1	7.5	8
10e	$CH_2CH{=}CH$	12	8.7	6.4	12

[a]Values represent the numerical average of at least two experiments, interassay variability was ±20%. [b]Values represent the numerical average of at least two experiments, interassay variability was ±10%. [c]% of remaining compound at 30 min.

Table 2. Arylsulfonyl SAR: Binding Affinities and Functional Activities of B1 Receptor Antagonists



compd	R	K_i [a] (nM) (human)	pK_B [b] (human)	pK_B [b] (mouse)	metabolic stability[c] (human)
21a	2,6-di-Me-4-OMe-Ph	0.3	9.7	8.2	57
21b	2-CF_3-Ph	2.8	9.2	nd	50
10d	2,4-di-Cl-3Me-Ph	2.1	9.1	7.5	8
21c	2-Me-4-OMe-Ph	5.5	8.6	6.9	91[d]
10f	2,3-di-Cl-Ph	3.1	8.8	7.2	nd
10g	2,6-di-Cl-Ph	4.2	8.5	7.2	nd
21d	2-Cl-4-OMe-Ph	4.3	8.5	7.3	64
21e	2-CN-Ph	63.5	8.1	6	87
21f	2-Me-3-Cl-Ph	6.8	8	nd	48
21g	2,6-di-F-Ph	142	7.6	6	87
21h	4-CF3O-Ph	85	7.5	nd	86

[a]Values represent the numerical average of at least two experiments; interassay variability was ±20%. [b]Values represent the numerical average of at least two experiments; interassay variability was ±10%. [c]% of remaining compound at 30 min. [d]% of remaining compound at 10 min.

cells (COS) expressing the recombinant human or mouse B_1 receptor. pK_B values were calculated from Schild plot obtained from concentration curves of des-Arg[10]-kallidin in the isolated human umbilical vein, mouse fundus.[12] Inflammation pain models have been previously described.[12] Hepatic microsomes were used as an in vitro surrogate for in vivo phase I metabolism. Metabolic stability of the different compounds was assessed at 1 μM for 10, 30, or 60 min at 37 °C in the presence of human or mouse liver microsomes. The permeability was measured using 50 μM compound on Caco2 cells.

DISCUSSION

Compound **3** was considered as a good starting point in terms of B1 activity. Initially, we chose to optimize the Gly-Gly linker while keeping other parts of the molecule unchanged. A complete series of compounds with various cyclic and linear hydrophobic linkers was synthesized. Table 1 shows only the most promising compounds. The presence of the ether linker in **10d**, a nanomolar antagonist (K_i = 2.1 nM), was particularly remarkable. Unfortunately this compound was poorly metabolically stable (8% remaining after 1 h at 1 μM on human microsomes) and showed some degree of cytochrome P450 2D6 (CYP 2D6) inhibition (63% at 5 μM). The main metabolites were hydroxylation of the arylsulfonyl group and N-demethylation of the sulfonamide.

The replacement of the 2,4-dichloro-3-methylphenyl group by a wide variety of different substituted aryl or heteroaryl groups was systematically investigated as a means for both improving metabolic stability and avoiding CYP inhibition. Sixty compounds were synthesized and evaluated on the B_1 receptor. No clear SAR could be drawn; it was just noticed that the most potent compounds have an ortho substituent such as methyl, chlorine, or trifluoromethyl. Calculations of interaction energy, using ab initio methods, were not able to explain SAR and were considered not enough accurate for describing these subtle effects (data not shown). A selection of active compounds is given in Table 2. These modifications led to highly potent compounds down to the subnanomolar range with improved metabolic stability and low CYP interaction (<50% at 5 μM on 2C9, 2D6, and 3A4). Compound **21a** was selected for evaluation in rodent pain models. Compound **21a** showed antihyperalgesic activity in rats after sc administration. Compound **21a** significantly reversed carrageenan-induced thermal hyperalgesia (57% at 3 mg/kg sc) and complete

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Freund's adjuvant-induced inflammatory pain (61% at 10 mg/kg sc) and produced at 10 mg/kg sc a reduction in both the phase I and phase II flinching responses induced by formalin in rats (52% and 46%, respectively).[12] Compound **21a** reduced bone cancer pain-related behaviors in mice after iv administration.[13] Unfortunately, **21a**, having a low oral bioavailability in mice (12%) and rats (0.3%) (Table 5), showed no activity after oral administration in rodent pain model. As this compound was stable on rodent microsomes (80% remaining after 30 min) and had a high exposure after sc administration, it seemed that the limiting factor of the pharmacokinetics (PK) was the permeability (P_{app} =1.3 × 10^{-6} cm/s).

The phenylimidazoline group was suspected to be responsible for the poor permeability. A Caco2 permeability screen was done using 124 analogues (disregarding their B1 activity) bearing or not the phenylimidazoline group. This study showed that unlike phenylimidazoline derivatives, non-phenylimidazoline ones had good permeability. At this step of the project all the work was focused on the replacement of the phenylimidazoline group. The replacement of the imidazoline group was guided by modeling studies. Su et al. have described a homology model of the BK B1 receptor based on the crystal structure of the bovine rhodopsin.[14]In this model, the imidazoline ring interacts with Glu273 and Asp291. Maigret et al. have constructed another homology model based on the crystal structure of bacteriorhodopsin (unpublished data). They found a different binding site with the imidazoline ring interacting with Glu95, His37, and Asn99 (Figure 3). This is

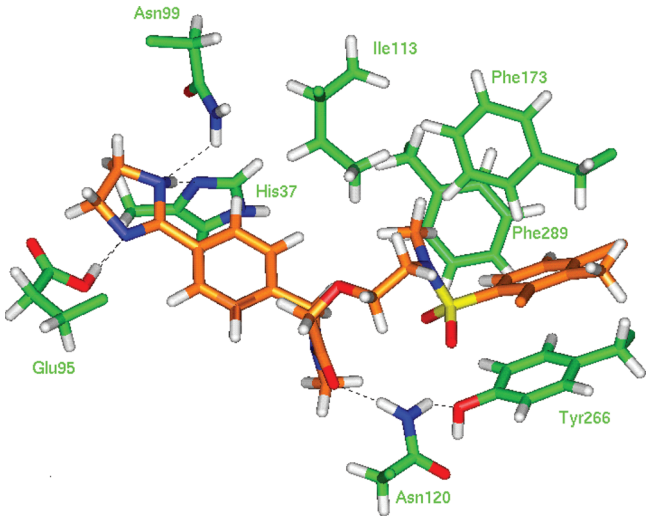
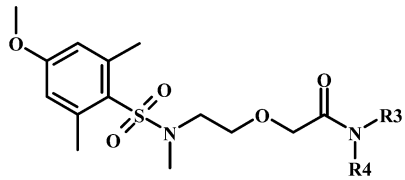
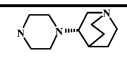
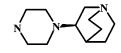
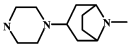
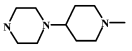
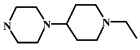
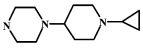
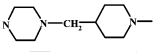
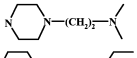
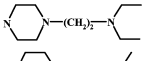
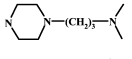


Figure 3. Homology model of BK B1 receptor with **10d** docked in the putative binding site.

supported by species activity differences. The residue His37 is present in rabbit (His36) but not in rat and mouse in which it is replaced by Tyr36 (rat) and Cys36 (mouse). Actually, our compounds were equipotent on human and rabbit and displayed approximately between 20- and 30-fold loss of activity in rat and mouse: for example, **21a** pK_B of 9.7 (human), 9.5 (rabbit), 8.2 (mouse), and 8.1 (rat). Our compounds were docked in both models; a better correlation between docking energy and activity was obtained with Maigret's model than with Su one. The imidazoline ring was replaced by a wide variety of H bond acceptor and/or donor containing groups (like imidazole, oxadiazole, pyrazole, oxazole, thiazole, triazole, amide groups, ether groups, acid derivatives, tetrazole, ...) likely to make hydrogen bond interactions with Asn99, Glu95, or His37. Unfortunately all these modifications led to inactive compounds. These results revealed that a basic group seemed to be mandatory for the activity. The phenylimidazoline group was replaced by several basic amino groups such as substituted piperazine, piperidine. These groups were chosen in order to improve physicochemical parameters such as pK_a, log *P*, and polar surface area (PSA). These new derivatives have calculated pK_a ranging from 8.5 to 10 (**21a** calculated pK_a = 9.9), reduced PSA (around 90 Å^2 compared to 109 Å^2 for **21a**), and improved calculated log *P* (down to 0.7 compared to 1.6 for **21a**). More than 70 compounds have been synthesized and evaluated on the human B_1 receptor. The replacement of the phenylimidazoline group by the piperazinoquinuclidine group led to the first potent non-imidazoline derivatives. Compound **12a** (*S* isomer) is more potent and metabolically stable than **12b** (*R* isomer) but without improvement of permeability (Table 3) and mouse bioavailability (Table 5). Among

Table 3. Piperazine SAR: Binding Affinities and Functional Activities of B1 Receptor Antagonists



Comp.	NR3R4	Ki[a] (nM) (human)	pK_B[b] (human)	Metabolic stability[c] (human)	Papp (10^{-6} cm/s) Caco2
12a		5.6	8.1	57	1.4
12b		34	7.2	26	1.9
12c		3.3	8.4	46	1.2
12d		1.6	8.4	77	1.2
12e		0.2	8.8	85	1.7
12f		0.8	8.5	6	11.8
12g		4.5	8.6	86	1.5
12h		106	7.4	84	2.1
12i		10	7.8	73	0.9
12j		5.8	7.9	67	0.1

[a]Values represent the numerical average of at least two experiments; interassay variability was ±20%. [b]Values represent the numerical average of at least two experiments; interassay variability was ±10%. [c] % of remaining compound at 30 min.

piperazine derivatives, constrained compounds (**12c–g**) are more potent than flexible ones (**12h–j**), especially **12d**, **12e**, and **12f** which have an affinity in the nanomolar to subnanomolar range with K_i of 1.6, 0.2, and 0.8 nM, respectively, and a functional activity with pK_B between 8.4 and 8.8. Compounds **12d** and **12e** are more stable on human microsomes than **21a** (77% and 85% remaining versus 57% after incubation at 1 μM for 30 min) with improved bioavailability (18% and 12% versus 0.3% in rat for **21a**) (Table 5) despite no improvement of permeability on Caco2 cells. Compound **12f**, which has a better permeability (Table 3)

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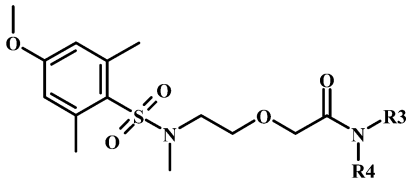
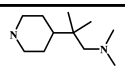
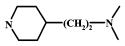
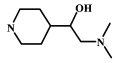
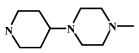
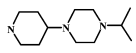
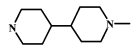
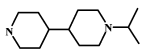
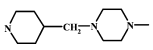
than **21a**, was not evaluated in PK because of its poor stability on microsomes. Piperidine derivatives were also prepared, leading to highly potent compounds (**12l** and **12o−q**) (Table 4) in nanomolar to subnanomolar range with K_i between 3.8 and 0.36 nM. Compounds **12o−q** have a moderate to low human metabolic stability (of minor level compared to potent piperazine analogues). Compound **12l** is stable on human microsomes (86% remaining after incubation at 1 μM for 30 min) and exhibits a bioavailability of 38% in mice and 19% in rats (Table 5). Unfortunately **12l** is a strong inhibitor of CYP450 2D6 and was not further evaluated.

Table 4. Piperidine SAR: Binding Affinities and Functional Activities of B1 Receptor Antagonists



Comp.	NR3R4	Ki[a] (nM) (human)	pK_B[b] (human)	Metabolic stability[c] (human)	Papp (10^{-6} cm/s) Caco2
12k		10	7.5	36	13.6
12l		3.5	8.2	86	0.5
12m		30	7.4	100	1.3
12n		9.5	7.5	68	0.1
12o		3.8	8.5	54	0.9
12p		0.7	9	23	2.6
12q		0.4	9.3	23	3
12r		4.7	8.1	81	4.8

[a]Values represent the numerical average of at least two experiments; interassay variability was ±20%. [b]Values represent the numerical average of at least two experiments; interassay variability was ±10%. [c] % of remaining compound at 30 min.

Compound **12d** was selected for further investigation. **12d** is selective over 65 receptors (including BK B2), 12 ion channels, and 7 enzymes (Table 6), has no interaction with CYP 450 (9 isoforms) (Table 7), and has a dog bioavailability of 43% (Table 5). As shown in Figure 4, **12d** orally given in rats at 30 mg/kg, 60 min before formalin injection, significantly reduced the amplitudes of both acute phase and late phase of the response by 61% and 54%, respectively, yielding an approximate ED_{50} of 30 mg/kg for the first and second phases. Moreover, **12d** significantly reversed carrageenan-induced mechanical hyperalgesia in a dose-dependent manner with a maximum activity obtained at 300 mg/kg, giving an approximate ED_{50} of 100 mg/kg (Figure 5) .The effect obtained with **12d** at 300 mg/kg was similar to that observed with morphine (8 mg/kg po) and stronger than that observed with diclofenac (20 mg/kg po). Compound **12d** was more effective in producing antinociceptive action against acute chemical stimulus than mechanical stimulus, which is in accordance with results from BK B_1 knockout (KO) mice.[6]

CONCLUSION

Starting from a nonselective BK B1/B2 receptor antagonist, we have identified a novel class of highly and selective potent BK B1 receptor antagonists. Unfortunately these compounds suffered from a lack of permeability. Design and synthesis of less basic compounds led to potent and selective compounds like **12d**, which has oral antihyperalgesic activity in animal.

EXPERIMENTAL SECTION

General. Reagents and solvents obtained from commercial suppliers are used without further purification unless otherwise stated. ^{1}H NMR spectra were recorded on a Bruker Avance spectrometer. Chemical shifts are reported in ppm (δ) and were calibrated using the undeuterated solvent resonance as internal standard. Melting points were determined on a hot stage apparatus and are uncorrected. All final compounds were purified to >95% purity as determined by Agilent HP1100 series or Waters ACQUITY high performance liquid chromatography (HPLC) with UV detection at 254 nm using one of the following methods. Method A: X-Terra C18 column (50 mm, 2.1 mm, 3.5 μm, 45 °C); mobile phase, A = H_2O with 0.05% TFA, B = CH_3CN with 0.05% TFA; gradient, 10−90% B (0.0−9.0 min); flow rate, 0.6 mL/min. Method B: Uptispher ODB column (50 mm, 2 mm, 3 μm, 45 °C); mobile phase, A = H_2O with 0.05% TFA, B = CH_3CN with 0.05% TFA; gradient, 10−90% B (0.0−9 min); flow rate, 0.6 mL/min. Method C: ACQUITY UPLC BEH C18 column (50 mm, 2.1 mm, 1.7 μm, 45 °C); mobile phase, A = H_2O with 0.1% CH_3CO_2H, B = CH_3CN with 0.1% CH_3CO_2H; gradient, 15−95% B (0.0−2.5 min); flow rate, 0.8 mL/min. Low resolution mass spectrometry (MS) data were obtained using a Waters ZQ mass spectrometer or a Waters SQD

Table 5. PK Profiles of Selected B1 Receptor Antagonists

	mouse[a]				rat[b]				dog[c]			
compd	F[d]	$T_{1/2}$[e]	Cl[f]	Vd[g]	F[d]	$T_{1/2}$[e]	Cl[f]	Vd[g]	F[d]	$T_{1/2}$[e]	Cl[f]	Vd[g]
21a	12	0.6	131	2.6	0.3	2.1	27.4	1.4				
12a	6.7	1.7	23.5	1								
12d	22	1	24.1	1.3	18	3.7	26	1.5	43	3.7	12.7	2.7
12e					12	1.6	66.7	3.8				
12g					16	2.8	32.5	3.5				
12k					3.8	1.6	19.2	0.5				
12l	38	1	25	0.5	19	1	18.8	0.79				
12n					6	6.5	13.1	0.6				
12p					12.3	1.3	17.9	0.6				

[a]Male Swiss mice (n = 3): 5 mg/kg po; 1 mg/kg iv. Interanimal variability was less than 20%. [b]Sprague−Dawley rats (n = 3): 10 mg/kg po; 1 mg/kg iv. Interanimal variability was less than 20%. [c]Beagle dogs (n = 3): 5 mg/kg po; 1 mg/kg iv. Interanimal variability was less than 20%. [d]F in % oral bioavailability. [e]Half-life in hours. [f]Cl in mL min^{-1} kg^{-1}. [g]Vd in L/kg.

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Table 6. Effect of 12d (5 μM) for a Range of Specific Receptor Binding and Cell Biology Assays[a]

receptor			receptor			receptor or assay		
A_1(h)	human	18	motilin	human	−16	VIP_1 (h) (VPAC1)	human	5
A_{2A}	human	1	M_1	human	15	V_{1a}	human	31
A_3	human	16	M_2	human	16	Ca^{2+} channel L		0
α_1 (nonselective)		0	M_3	human	8	(DHP site)		
α_{2A}	human	−4	M_4	human	4	Ca^{2+} channel L		3
α_{2B}		0	M_5	human	4	(diltiazem site)		
α_{2C}	human	36	NK_1	human	10	Ca^{2+} channel L		10
β_1	human	18	NK_2	human	−9	(verapamil site)		
NE transporter	human	2	NK_3	human	8	Ca^{2+} channel N		5
BZD (central)		16	Y_1	human	11	K^+_{ATP} channel		2
bombesine (nonselective)		6	NT_1 (NTS1)	human	−3	K^+_V channel		2
bradykin (B_2)	human	26	N (neuronal) (BGTX-insensitive)		19	SK^+_{Ca} channel		6
CGRP	human	−4	N (neuronal) (BGTX-sensitive)		3	Na^+ channel (site 1)		−8
CB_1	human	−4	δ	human	5	Na^+ channel (site 2)		3
CB_2	human	8	κ		−5	Cl^- channel		−2
CCKB (CCK2)		18	μ	human	18	COX_1	human	18
CRF_1		−21	OEL1	human	1	COX_2	human	−18
Dl	human	−5	PACAP		5	PGE_2 secretion	human	25
D2	human	4	PAF		17	PGI_2 secretion	human	26
GABA (nonselective)		−2	PCP		−3	TXB_2 secretion	human	17
galanin (nonselective)		8	TXA2/PGH2 (h) (TP)	human	−20	NOS inducible		8
AMPA		23	P2X		5	NOS constitutive	human	10
kainate		31	P2Y		−3			
NMDA		8	$5\text{-}HT_{1A}$	human	4			
glycine (strychnine-sensitive)		−1	$3\text{-}HT_{1B}$		1			
glycine (strychnine-insensitive)		−4	$3\text{-}HT_3$	human	1			
H_1 (central)		11	$3\text{-}HT_7$	human	−23			
I_2 (central)		9	3-HT transporter	human	3			
MC_3	human	−3	σ (nonselective)		29			
MC_4	human	8	glucocorticoid	human	−8			
ML_1		6						
MAO-A		2						

[a]The results are expressed as percent inhibition of control specific binding or control activity (mean values; n = 2).

Table 7. Cytochromes P450 inhibition of 12d[a]

CYP450 (% Inhibition at 5 μM)								
1A2	2A6	2B6	2C8	2C9	2C19	2D6	2E1	3A4
−4	17	8	−4	−19	0	2	8	−2

[a]Values are the mean, n = 3.

mass spectrometer operated in electrospray ionization (ESI) mode (positive or negative)

2,4-Dichloro-3,*N*-dimethyl-*N*-(2-hydroxyethyl)-benzenesulfonamide, 6d. A solution of 2.8 g (37.8 mmol) of 2-methylaminoethanol is prepared in 120 mL of dichloromethane, and 7.5 g (28.9 mmol) of 2,4-dichloro-3-methylbenzenesulfonyl chloride in solution in 30 mL of dichloromethane, and 10.5 mL of triethylamine are added. The reaction mixture is kept under agitation for 15 h at ambient temperature and then washed with a solution of 1 N hydrochloric acid, with a solution of sodium bicarbonate, and then with water. The organic phase is dried over magnesium sulfate and then concentrated under reduced pressure. The residue was used directly in the next step.

***N*-(2-Hydroxyethyl)-4-methoxy-*N*,2,6-trimethylbenzenesulfonamide, 6h.** Compound **6h** was prepared as described for **6d** using 2,6 dimethyl-4-methoxybenzenesulfonyl chloride and 2-methylaminoethanol (colorless oil, 99%). ^{1}H NMR (300 MHz, DMSO) δ: 6.80 (s, 2H), 4.70 (t, 1H), 3.80 (s, 3H), 3.48 (q, 2H), 3.09 (t, 2H), 2.69 (s, 3H), 2.54 (s, 6H).

[2-[[(2,4-Dichloro-3-methylphenyl)sulfonyl]methylamino]-ethoxy]acetic Acid 1,1-Dimethylethyl Ester, 7d. A solution of 100 mg (0.335 mmol) of compound **6d** is prepared in 4 mL of toluene, and 30 mg (0.111 mmol) of tetrabutylammonium chloride and 4 mL of a 35% aqueous solution of sodium hydroxide are added. The reaction medium is cooled to 10 °C, and an amount of 98 mg (0.5 mmol) of *tert*-butyl bromoacetate is then added. The mixture is stirred at ambient temperature for 30 min, then hydrolyzed over ice−water. The mixture obtained is extracted with toluene, and the organic phase obtained is dried over magnesium sulfate and concentrated under reduced pressure. The expected product is thus obtained (colorless oil, 87%). ^{1}H NMR (300 MHz, DMSO) δ: 7.84 (d, 1H), 7.63 (d, 1H), 3.93 (s, 2H), 3.59 (t, 2H), 3.39 (t, 2H), 2.91 (s, 3H), 2.49 (s, 3H), 1.41 (s, 9H).

[2-[[(4-Methoxy-2,6-dimethylphenyl)sulfonyl]methylamino]-ethoxy]acetic Acid 1,1-Dimethyl Ethyl Ester, 7h. Compound **7h** was prepared as described for **7d** using **6h** (colorless oil, 94%). ^{1}H NMR (250 MHz, DMSO) δ: 6.80 (s, 2H), 3.89 (s, 2H), 3.80 (s, 3H), 3.56 (t, 2H), 3.21 (t, 2H), 2.71 (s, 3H), 2.53 (s, 3H), 1.41 (s, 9H).

[2-[[(2,4-Dichloro-3-methylphenyl)sulfonyl]methylamino]-ethoxy]acetic Acid, 8d. A solution of 1.45 g (3.51 mmol) of the ester **7d** is prepared in 15 mL of tetrahydrofuran (THF), and 297 mg (7.02 mmol) of lithium hydroxide and 30 mL of water are added. The reaction mixture is stirred for 15 h at ambient temperature. The THF is drawn off under reduced pressure, and an amount of 50 mL of water is added to the residual aqueous phase. The aqueous phase is acidified with the aid of 1 N hydrochloric acid solution and extracted with ethyl acetate. The organic phase obtained is washed with water, dried over magnesium sulfate, and concentrated under reduced pressure. An amount of 1.24 g of the expected acid is thus obtained (colorless oil, 99%). ^{1}H NMR (300 MHz, DMSO) δ: 12.5 (broad s, 1H), 7.86 (d,

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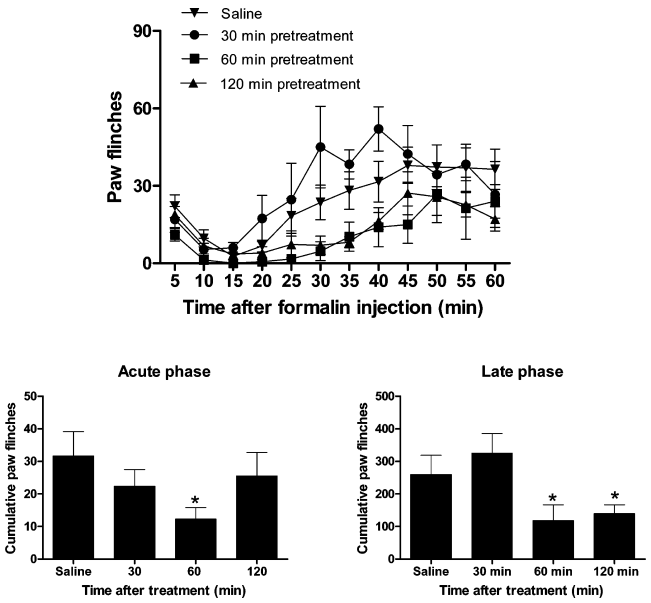

Figure 4. Effect of **12d** (30 mg/kg po) on formalin induced flinching in rats. Rats received an intraplantar injection (50 μL) of formalin (2%) into one hind paw. The duration of flinching of the injected paw was recorded continuously immediately thereafter for 60 min. The nociceptive response is divided into two phases, the acute one between 0 and 10 min and the late one between 15 and 60 min. Values are the mean ± SEM; n = 6 rats/group; analysis of variance followed by Student's t test, (*) $P < 0.05$ vs saline.

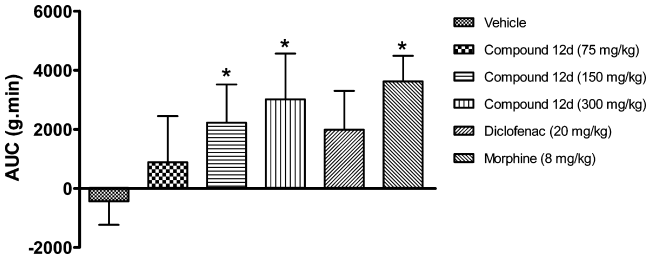

Figure 5. Effect of oral administration of **12d** on the vocalization thresholds due to paw pressure in carrageenan-treated rats. Effect of oral administration of compound **12d** on the vocalization thresholds due to paw pressure in carrageenan-treated rats. The results are expressed by the mean ± SEM of the area under the time-course curves (AUC) of the variations (postdrug − predrug values of vocalization thresholds), calculated by the trapezoidal method. Rats were treated with vehicle, compound **12d** (75, 150, and 300 mg/kg), diclofenac (20 mg/kg), or morphine (8 mg/kg) (n = 6−11 per group). Analysis of variance followed by Student's t test: (*) $p < 0.05$ vs vehicle.

1H), 7.64 (d, 1H), 4.02 (s, 2H), 3.61 (t, 2H), 3.40 (t, 2H), 2.91 (s, 3H), 2.51 (s, 3H).

[2-[[(4-Methoxy-2,6-dimethylphenyl)sulfonyl]methylamino]-ethoxy]acetic Acid, 8h. Compound **8h** was prepared as described for **8d** using **7h** (white solid, 95%). Mp 82 °C. ^{1}H NMR (300 MHz, DMSO) δ: 6.80 (s, 2H), 3.94 (s, 2H), 3.80 (s, 3H), 3.56 (t, 2H), 3.22 (t, 2H), 2.70 (s, 3H), 2.53 (s, 6H).

2-[4-[[[[2-[[(2,4-Dichloro-3-methylphenyl)sulfonyl]-methylamino]ethoxy]acetyl]methylamino]methyl]phenyl]-4,5-dihydro-1*H*-imidazole-1-carboxylic Acid 1,1-Dimethylethyl Ester, 9d. A solution of 200 mg (0.565 mmol) of the acid **8d** is prepared in 15 mL of dichloromethane. Then 120 mg of EDCI and 90 mg of HOAT are added. The mixture is stirred at ambient temperature for 20 min, and an amount of 163 mg (0.565 mmol) of the amine **26** in solution in 3 mL of dichloromethane is added. The reaction mixture is kept under stirring at ambient temperature for 15 h and diluted with 40 mL of dichloromethane. This organic phase is washed with water, dried over magnesium sulfate, and concentrated under reduced pressure. The oily product obtained is purified by chromatography on silica gel, eluting with a mixture of dichloromethane/methanol (98/2, v/v). An amount of 288 mg of the expected compound is thus obtained as a colorless oil (81%). ^{1}H NMR (250 MHz, DMSO) δ: 7.87 (t, 1H), 7.62 (m, 1H), 7.44 (m, 2H), 7.24 (d, 2H), 4.51 (s, 2H), 4.19 (d, 2H), 3.83 (m, 4H), 3.62 (m, 2H), 3.43 (m, 2H), 2.85 (m, 6H), 1.18 (s, 9H).

2-[2-[[(2,4-Dichloro-3-methylphenyl)sulfonyl]methylamino]-ethoxy]-*N*-[[4-(4,5-dihydro-1*H*-imidazol-2-yl)phenyl]methyl]-*N*-methylacetamide Trifluoroacetate, 10d. A mixture of 240 mg of the compound **9d**, 5 mL of trifluoroacetic acid, 5 mL of dichloromethane, and 41 mg of anisole is prepared, and this mixture is kept under agitation for 15 h at ambient temperature. The solvents are drawn off under reduced pressure and then in the presence of toluene. The residual oil is stirred with isopropyl ether which is then removed. The oily residue is taken up with pure water. The solution is filtered, and the filtrate is lyophilized. An amount of 240 mg of the desired product is thus obtained as a cotton-like white solid (98%). Mp 75 °C. ^{1}H NMR (300 MHz, DMSO) δ: 10.48 (broad, 2H), 7.87 (m, 3H), 7.63 (m, 1H), 7.49 (d, 2H), 4.60 (s, 2H), 4.26 and 4.15 (s, 2H), 4.01 (s, 4H), 3.64 and 3.62 (t, 2H), 3.39 (m, 2H), 2.91 and 2.86 (s, 3H), 2.90 and 2.78 (s, 3H), 2.50 (s, 3H). Analytical HPLC method C: t_R = 1.12 min (97.5%). MS (ESI, [M + H]$^+$), m/z 527.3.

4-Methoxy-*N*,2,6-trimethyl-*N*-[2-[2-[4-(1-methyl-4-piperidinyl)-1-piperazinyl]-2-oxoethoxy]ethyl]benzenesulfonamide Bistrifluoroacetate, 12d. 500 mg (1.51 mmol) of acid **8h**, 319 mg (1.65 mmol) of EDCI, 2.26 mg (1.65 mg) of HOAT, and 233 μL of triethylamine are mixed in 10 mL of DMF, and this reaction mixture is kept under agitation at ambient temperature for 30 min. An amount of 314 mg (1.61 mmol) of 4-(1-methyl-4-piperidinyl)-1-piperazine is then added, and stirring is carried out for 20 h at ambient temperature. The reaction mixture is poured onto ice−water and extracted with dichloromethane. The organic phase is dried over magnesium sulfate and concentrated under reduced pressure. The oily product obtained is purified by chromatography on silica gel, eluting with a mixture of toluene/isopropanol (95/5, v/v). The basic compound obtained is salified with trifluoroacetic acid to give 877 mg of a colorless paste (74%). ^{1}H NMR (300 MHz, CD_3CN) δ: 6.74 (s, 2H), 4.11 (s, 2H), 3.81 (s, 3H), 3.80 (m, 4H), 3.77 (d, 2H), 3.64 (m, 2H), 3.55 (m, 1H), 3.34 (t, 2H), 3.26 (m, 4H), 3.01 (m, 2H), 2.79 (s, 3H), 2.74 (s, 3H), 2.57 (s, 6H), 2.29 (m, 4H). Analytical HPLC method B: t_R = 3.02 min (99.3%). MS (ESI, [M + H]$^+$), m/z 497.

4-Methoxy-*N*,2,6-trimethyl-*N*-[2-[2-[4-(4-methyl-1-piperazinyl)-1-piperidinyl]-2-oxoethoxy]ethyl]benzenesulfonamide Fumarate, 12n. Compound **12n** was prepared as described for **12d** using 4-(4-methyl-1-piperazinyl)-1-piperidine (white powder, 95%). Mp 90 °C. ^{1}H NMR (300 MHz, CD3CN) δ: 6.77 (s, 2H), 6.60 (s, 2H), 4.02 (s, 2H), 3.80 (s, 3H), 3.54 (t, 2H), 3.43 (m, 2H), 3.30 (m, 2H), 3.26 (t, 2H), 3.14 (m, 2H), 2.73 (s, 3H), 2.56 (s, 6H), 2.47 (m, 6H), 2.45 (s 3H), 2.38 (m, 1H), 1.90 (m, 2H), 1.68 (m, 2H). Analytical HPLC method B: t_R = 3.71 min (97.6%). MS (ESI, [M + H]$^+$), m/z 497.

[2-[[(1,1-Dimethylethoxy)carbonyl]methylamino]ethoxy]-acetic Acid 1,1-Dimethylethyl Ester, 15. Compound **15** was prepared as described for **7d** starting from (2-hydroxyethyl)-methylcarbamic acid *tert*-butyl ester (yellow oil, 99%). ^{1}H NMR (250 MHz, DMSO) δ: 3.96 (s, 2H), 3.52 (t, 2H), 3.31 (t, 2H), 2.81 (s, 3H), 1.42 (s, 9H), 1.38 (s, 9H).

[2-(Methylamino)ethoxy]acetic Acid Trifluoroacetate, 16. A solution of 27.8 g (96 mmol) of **15** is prepared in 230 mL of dichloromethane. An amount of 20 mL of TFA is added and the mixture stirred overnight at room temperature. The mixture is concentrated under reduced pressure and dried at 45 °C under reduced pressure for 1 h to give the desired product as a yellow oil (99%). ^{1}H NMR (250 MHz, DMSO) δ: 8.50 (broad s, 1H), 4.09 (s, 2H), 3.70 (m, 2H), 3.11 (m, 2H), 2.60 (m, 3H).

[2-[Methyl[(phenylmethoxy)carbonyl]amino]ethoxy]acetic Acid, 17. A solution of 25 g (101 mmol) of **16** is prepared in 400 mL

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of dichloromethane. Then 35.2 mL (25 mmol) of triethylamine and, dropwise, 15.5 mL of benzyl chloroformate are added at 0 °C. The reaction mixture is stirred for 5 h at ambient temperature. The reaction mixture is hydrolyzed over 200 mL of ice−water and 50 mL of 1 N hydrochloric acid. The separated organic phase is washed with water, then dried over magnesium sulfate and concentrated under reduced pressure. The crude oily product is purified by chromatography on silica gel, eluting with a mixture of toluene/isopropanol/aqueous ammonia (9/1/0.1, v/v/v). An amount of 13.7 g of the desired product is thus obtained as a colorless oil (51%). ^{1}H NMR (250 MHz, DMSO) δ: 7.33 (m, 5H), 5.06 (s, 2H), 4.10 (s, 2H), 3.58 (t, 2H), 3.49 (m, 2H), 2.90 (d, 3H).

2-[4-(2,8-Dimethyl-3,9-dioxo-11-phenyl-5,10-dioxa-2,8-diazaundec-1-yl)phenyl]-4,5-dihydro-1*H*-imidazole-1-carboxylic Acid 1,1-Dimethylethyl Ester, 18. Compound **18** was prepared as described for **9d** using **17** (yellow oil, 62%). ^{1}H NMR (250 MHz, DMSO) δ: 7.42−7.22 (m, 9H), 5.05 (s, 2H), 4.51 (s, 2H), 4.21 (s, 2H), 3.84 (m, 4H), 3.58 (m, 2H), 3.45 (m, 2H), 2.88 (m, 6H), 1.17 (s, 9H).

4,5-Dihydro-2-[4-[[methyl[[2-(methylamino)ethoxy]acetyl]amino]methyl]phenyl]-1*H*-imidazole-1-carboxylic Acid 1,1-Dimethylethyl Ester, 19. A solution of 11.7 g (22 mmol) of **18** is prepared in 200 mL of methanol. An amount of 1.2 g of Pd/C 10% is added, and the mixture is hydrogenated at room temperature and atmospheric pressure for 3 h. The catalyst is eliminated by filtration, and the filtrate is concentrated under reduce pressure. The crude product is purified by chromatography on NH_2 silica gel, eluting with a mixture of dichloromethane/MeOH (99/1, v/v). An amount of 6 g of the desired product is thus obtained as a colorless oil (91%). ^{1}H NMR (250 MHz, DMSO) δ: 7.41 (d, 2H), 7.26 (d, 2H), 4.53 (d, 2H), 4.20 (d, 2H), 3.84 (m, 4H), 3.50 (m, 2H), 2.86 (s, 3H), 2.63 (m, 2H), 2.26 (d, 3H), 1.18 (s, 9H).

4,5-Dihydro-2-[4-[[[[2-[[(4-methoxy-2,6-dimethylphenyl)sulfonyl]methylamino]ethoxy]acetyl]methylamino]methyl]phenyl]-1*H*-imidazole-1-carboxylic Acid 1,1-Dimethylethyl Ester, 20a. A solution of 100 mg (0.24 mmol) of amine **19** is prepared in 3 mL of dichloromethane. Then 34 μL of Et_3N and 52 mg (0.22 mmol) of 4-methoxy-2,6-dimethylphenylsulfonyl chloride are added, and the mixture is stirred at room temperature for 2 h. The mixture is poured onto water and extracted with dichloromethane. The organic phase is dried on magnesium sulfate and concentrated under reduced pressure. The crude product is purified by chromatography on silica gel, eluting with a mixture of dichloromethane/MeOH (99/1, v/v). An amount of 99 mg of the desired product is thus obtained as a colorless oil (74%). ^{1}H NMR (300 MHz, DMSO) δ: 7.43 (m, 2H), 7.22 (d, 2H), 6.80 (s, 2H), 4.50 (s, 2H), 4.16 and 4.13 (s, 2H), 3.84 (m, 4H), 3.79 (s, 3H), 3.57 (m, 2H), 3.26 (m, 2H), 2.80 and 2.76 (s, 3H), 2.71 and 2.68 (s, 3H), 2.53 (s, 6H), 1.18 (s, 9H).

***N*-[[4-(4,5-Dihydro-1*H*-imidazol-2-yl)phenyl]methyl]-2-[2-[[(4-methoxy-2,6-dimethylphenyl)sulfonyl]methylamino]-ethoxy]-*N*-methylacetamide Trifluoroacetate, 21a.** Compound **21a** was prepared as described for **10d** using **20a** (pasty solid, 99%). ^{1}H NMR (250 MHz, DMSO) δ: 10.48 (broad s, 2H), 7.89 (d, 2H), 7.48 (d, 2H), 6.79 (s, 2H), 4.59 (s, 2H), 4.15 (d, 2H), 4.01 (s, 4H), 3.79 (s, 3H), 3.59 (m, 2H), 3.24 (m, 2H), 2.71 (m, 6H), 2.53 (s, 6H). Analytical HPLC method A: t_R = 3.39 min (99.6%). MS (ESI, $[M + H]^+$), m/z 503.

Competition Binding Assay to the Human or Mouse Recombinant B1 Receptor. HEK 293 cells stably expressing the human[15] or mouse B1 receptor were grown in Dulbecco's modified Eagle's medium containing 4.5 g/L glucose, 1% Glutamax (v/v), 1% nonessential amino acid (v/v), 1 mM sodium pyruvate, 100 mg/mL penicillin, 100 mg/mL streptomycin, and 10% fetal bovine serum. HEK 293 cells were rinsed twice with Ca^{2+}/Mg^{2+}-free ice-cold PBS and scraped from the dishes with a rubber policeman in 5 mL of binding buffer [25 mM 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino}ethanesulfonic acid (TES), 1 mM 1,10-phenanthroline, 140 mg/mL bacitracin, and 0.1% bovine serum albumin at pH 7.4]. They were homogenized with a Polytron (Kinematica, Lucerne, Switzerland) and centrifuged at 40000*g* for 20 min. The pellet was then resuspended in binding buffer and stored in liquid nitrogen. Binding experiments were performed at room temperature with a 20−40 μg of membrane protein/assay in binding buffer. For saturation experiments, [^{3}H]des-Arg10-KD (0.1−5 nM) was incubated for 1 h in a final volume of 500 μL. The assay was terminated by filtration on Whatman GF/B filters presoaked for 2 h in 0.1% polyethyleneimide (w/v). Filters were rinsed three times with 5 mL of ice-cold 50 mM TES, and the radioactivity was determined by liquid scintillation counting in 5 mL of Optima Gold (Packard, Rungis, France). Nonspecific binding was determined in the presence of 10 μM des-Arg10-kallidin. Competition binding experiments were carried out in the presence of [^{3}H]des-Arg10-kallidin at a dose equal to the KD in competition with various concentrations of compound. All assays were carried out in duplicate. Protein concentration was measured by the method of Bradford.[16]

Competition Binding Assay to the Human Recombinant B2 Receptor. Chinese hamster ovary (CHO) K1 cells expressing the human B2 receptor (Hess et al., 1992) were maintained in Ham's F12 medium containing 10% fetal calf serum, 4.5 g/L glucose, 100 mg/L streptomycin, and 105 units/L penicillin. CHO cells were rinsed twice with Ca^{2+}/Mg^{2+}-free ice-cold phosphate-buffered saline and scraped from the dishes with a rubber policeman in 5 mL of binding buffer (25 mM TES, 1 mM 1,10-phenanthroline, 140 mg/mL bacitracin, and 0.1% bovine serum albumin) at pH 6.8. They were homogenized with a Polytron (Kinematica) and centrifuged at 40000*g* for 20 min. The pellet was then resuspended in binding buffer and stored in liquid nitrogen. Binding experiments were performed at room temperature with 20−40 μg of membrane protein/assay in binding buffer. For saturation experiments, [^{3}H]bradykinin (0.1−5 nM) was incubated for 90 min in a final volume of 500 μL. The assay was terminated by filtration on Whatman GF/B filters presoaked for 2 h in 0.1% polyethyleneimide (w/v). Filters were rinsed three times with 5 mL of ice-cold 50 mM TES, and the radioactivity was determined by liquid scintillation counting in 5 mL of Optima Gold (Packard). Nonspecific binding was determined in the presence of 10 μM bradykinin. Competition binding experiments were carried out in the presence of [^{3}H]bradykinin at a dose equal to the KD in competition with various concentrations of compound for 90 min at room temperature. All assays were carried out in duplicate. Protein concentration was measured by the method of Bradford.[16]

Human Umbilical Vein. With the approval of the Ethical Committee of Clinique Ste Marthe (Dijon, France), human umbilical cords were collected postdelivery and immediately placed in Krebs' solution of the following composition: 119 mM NaCl, 4.7 mM KCl, 1.5 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 5.5 mM glucose, and 0.026 mM EDTA. Umbilical vein rings (3−4 mm in length) were incubated overnight in 20 mL jacketed organ baths containing Dulbecco's modified Eagle's cell culture medium supplemented with 1% fetal calf serum and penicillin (10 IU/mL)−streptomycin (10 μg/mL) maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2 to induce kinin B1 receptor. Then they were set up in 8 mL jacketed organ baths containing Krebs' solution maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2. Strips were left unstretched for 1 h, during which the bath fluid was changed every 15 min with fresh solution. Strips were then stretched to 1 g. All rings were contracted by KPSS (Krebs' solution in which NaCl was replaced by KCl) to assess their contractility. After the samples were washed twice with normal Krebs' solution and returned to baseline, captopril (10 μM), DL-thiorphan (1 μM), mepyramine (1 μM), atropine (1 μM), indomethacin (3 μM), NG-nitro-L-arginine (30 μM), and nifedipine (0.1 μM) were added into the organ bath. After 30 min vehicle or compound was injected into the bath, and after 30 min of incubation concentration−response curves to des-Arg10-kallidin were obtained. At the end of the experiments, after repetitive washes, the baseline level was reestablished and the maximal contraction of each vein segment was elicited by adding U46619 (1 μM), a thromboxane A2 mimetic. Each ring was used for a single concentration−response curve. The contractile responses to agonists were expressed as a percentage of the maximal contraction to U46619.

Mouse Fundus. Male Swiss mice weighing 25−35 g were sacrificed by a CO_2 intoxication, and the stomach was removed and

immediately placed in Krebs' solution of the following composition: 119 mM NaCl, 4.7 mM KCl, 1.5 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 5.5 mM glucose, and 0.026 mM EDTA. The fundus was dissected free, and segments were suspended under a resting tension of 1 g in 8 mL jacketed organ baths containing Krebs' solution maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2. After a 4 h resting period, the maximal contraction was measured in each segment by adding carbachol (10 μM). After the samples were washed twice with Krebs' solution and returned to baseline, captopril (10 μM), DL-thiorphan (1 μM), mepyramine (1 μM), and indomethacin (3 μM) were added to the organ bath. After 30 min, vehicle or compound was applied to the organ bath. This mixture was incubated for 30 min, and concentration−response curves to des-Arg10-kallidin were obtained. Each segment was used for a single concentration−response curve. The contractile responses to des-Arg10-kallidin were expressed as a percentage of the maximal contraction to carbachol.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures for all intermediates **5a**, **6f,g**, **7a−c,e−g**, **8a−c,e−g**, **9a−c,e−h**, **10a−c,e−g**, **12a−c,e−m,o−r**, **20b−h**, **21b−h**, and **23−26**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

ADME, absorption, distribution, metabolism, and excretion; BK, bradykinin; COS, monkey kidney fibroblast cells; CYP, cytochrome P450; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; HEK293, human endothelium kidney 293; HOAT, 1-hydroxy-7-azabenzotriazole; PSA, polar surface area; SAR, structure−activity relationship; TM, transmembrane spanning domain

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