Journal of Medicinal Chemistry Perspective

J. Med. Chem. **2010,** *53,* 5383–5399 **5383**
DOI: 10.1021/jm1000776

Bradykinin B1 Receptor Antagonists as Potential Therapeutic Agents for Pain

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Received January 18, 2010

Introduction

The Kinin Family. Kinins are naturally occurring vasoactive peptide hormones, which are known to be important mediators of a variety of biological effects, including cardiovascular homeostasis, inflammation, and nociception. The kinin family includes bradykinin (**1**, BK[a]), kallidin (**2**, Lys-BK), and their des-Arg metabolites (**3**, des-Arg^9-BK, and **4**, Lys-des-Arg^9-BK), which are produced by cleavage of the C-terminal arginine of **1** and **2**, respectively, by carboxypeptidases (Figure 1).[1] First isolated in 1949, **1** is a positively charged nonapeptide.[2] It is noteworthy that **1** was the first biologically active peptide reported to have been assembled by Merrifield's solid phase peptide synthesis.[3] Kinins originate from kininogens, which are the circulating glycoproteins synthesized primarily by the liver. The cleavage of kininogens by proteolytic enzymes known as kallikreins in either plasma or tissue produces the kinins **1** and **2** (Figure 1).[4] The kinins then interact with two G-protein-coupled receptors, termed B1 and B2 receptors (B1R and B2R).[5] Kinins **1** and **2** are potent (subnanomolar) agonists of the B2R and relatively short-lived (30 s or less), whereas the biological action of the relatively abundant metabolites, **3** (DABK) and **4** (Lys-DABK), is mediated by the B1R. The major degradation pathways of **1** are the aminopeptidase P cleavage of N-terminal arginine to generate des-Arg^1-BK and the inactivation through kininase II or angiotensin-converting enzyme (ACE), which produces primarily des-(Phe^8, Arg^9)-BK. Neither metabolite retains significant activity for either receptor type. As components of several classical signal transduction pathways, kinins and their receptors are primarily linked to the activation of phospholipase C, which causes intracellular Ca^{2+} mobilization by inositol 1,4,5-triphosphate.[6] Kinin **1**-induced release of secondary mediators, such as nitric oxide and prostaglandin I_2 from endothelial cells, is involved in endothelial-dependent vasorelaxation. By blocking the major degradation pathway of **1**, ACE inhibitors have helped to identify the cardioprotective role of kinins and the therapeutic potential of promoting the activities of the natural kinins.[7]

Involvement of Kinin Receptors in Disease. Overproduction of kinins under pathophysiological conditions is implicated in a number of disorders, including pain, inflammation, hypotension, asthma, colitis, pancreatitis, rhinitis, sepsis, and rheumatoid arthritis.[8,9] Therefore, antagonists of BK receptors may offer a novel approach to the treatment of these disorders, among which perhaps the most promising area is in the treatment of pain. The role of kinins in mediating nociceptive responses in animals has been studied for over 2 decades and was elucidated by the observation that injection of **1** into the knee of rats elicited a hyperalgesic state.[10] The specific role of B1R and B2R in pain-related processes is further informed by their expression patterns, as both are expressed by vascular cells,[11] nonvascular smooth muscle, fibroblasts,[12] unmyelinated afferent neurons, and various tumor cells,[13] though at different levels.[14] The B2R is ubiquitously and constitutively expressed, whereas the B1R is expressed at a very low level in healthy tissues but is rapidly induced following tissue injury. For example, the B1R is up-regulated in certain inflammatory states, such as septic shock, rheumatoid arthritis, and airway inflammation.[15] However, there is also evidence that the B1R is constitutively expressed in the central nervous system (CNS) of humans and rats.[16,17] With respect to functional regulation, the B2R appears to be rapidly phosphorylated, internalized, and thus desensitized to its agonists **1** and **2**. The ligand-induced B2R phosphorylation occurs at specific serine and threonine residues near the C-terminus. Conversely, the B1R lacks any C-terminal serine or threonine residues that are conserved across species. Thus, the B1R is less prone to desensitization, as it is not phosphorylated and internalized after agonist stimulation.[18] The difference in the expression pattern and in the extent of desensitization of B1R and B2R may contribute to their rather distinct roles in pain and in inflammatory processes. The B2R is involved in the early phases or acute state of inflammatory pain, whereas the B1R participates prominently in the establishment and maintenance of chronic pain.[19]

Therefore, B1R antagonists would be targeted to chronic inflammatory pain, whereas B2R antagonists would be most likely used to treat acute pain. Besides being capable of reversing inflammatory hyperalgesia, B2R antagonists are useful in the treatment of angioedema, a rare but potentially life-threatening side effect of ACE inhibitors. As part of

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[a] Abbreviations: BK, bradykinin; Lys-BK, kallidin; B1R, B1 receptor; B2R, B2 receptor; DABK, des-Arg^9-bradykinin; Lys-DABK, Lys-des-Arg^9-bradykinin; ACE, angiotensin-converting enzyme; CNS, central nervous system; KO, knockout; IL, interleukin; CFA, complete Freund's adjuvant; LPS, lipopolysaccharide; PSNL, partial sciatic nerve ligation; CCI, chronic constriction injury; MRC5, human fetal lung fibroblast; HEK, human embryonic kidney; FLIPR, fluorescence imaging plate reader; CHO, Chinese hamster ovary; PD, pharmacodynamic; PK, pharmacokinetic; NSAIDs, nonsteroidal anti-inflammatory drugs; HAE, hereditary angioedema; sc, subcutaneous; iv, intravenous; po, oral; ip, intraperitoneal; DHQ, dihydroquinoxalinone; TM, transmembrane; t.i.d., three times a day; P-gp, P-glycoprotein; MDR, multidrug resistance; hERG, human ether-à-go-go related gene; MDCK, Madin−Darby canine kidney; IDR, idiosyncratic drug reactions; CYP2C75, cytochrome P450 2C75; PXR, pregnane X receptor; AGM, African green monkey.

Published on Web 04/06/2010

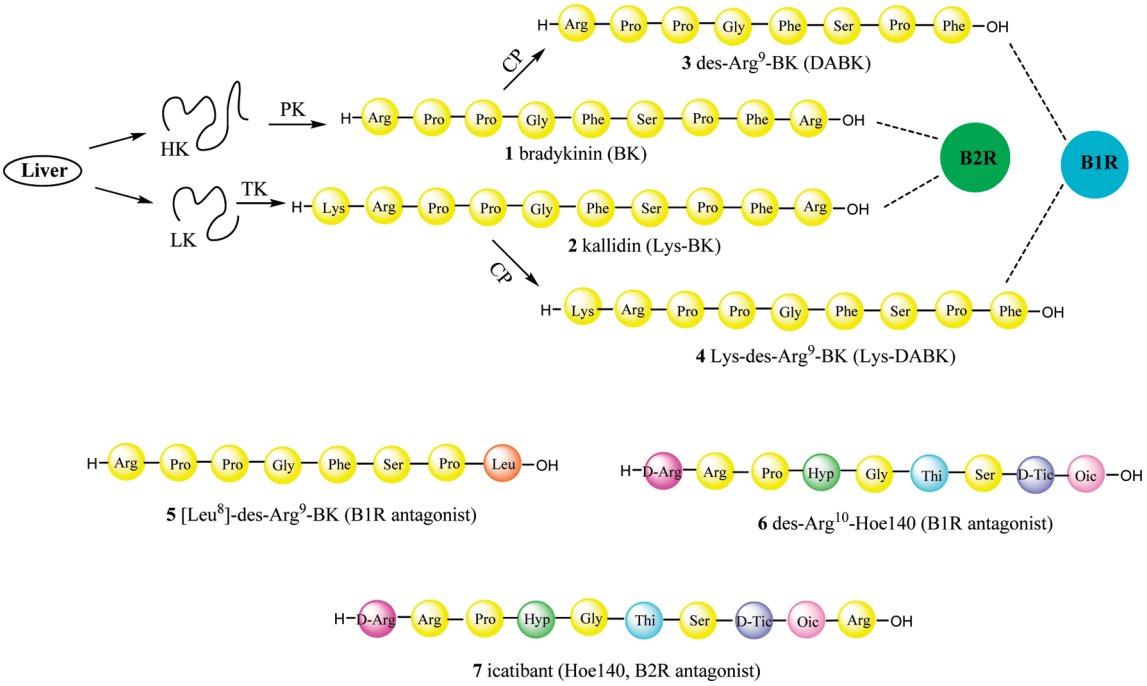


Figure 1. Schematic representation of the kallikrein−kinin system and B1R/B2R peptide antagonists: (HK) high-molecular-mass kininogen (88−120 kDa); (LK) low-molecular-mass kininogen (50−68 kDa); (PK) plasma kallikrein; (TK) tissue kallikrein; (CP) carboxypeptidases; (Hyp) 4-hydroxyproline; (Thi) 2-amino-3-thiophen-2-ylpropionic acid; (Tic) D-2,3,4-tetrahydroisoquinoline-3-carboxylic acid; (Oic) L-(3a*S*,7a*S*)-octahydroindole-2-carboxylic acid.

research efforts exploring the B2R as a drug target for pain, emergent evidence suggested that the B2R also functions in a cardioprotective role.[20] The B2R knockout (KO) mice exhibited salt-sensitive hypertension, demonstrating an important role for the B2R in cardiovascular function.[21] The B2R agonists may be beneficial in the treatment of cardiovascular disorders, such as congestive heart failure, hypertension, and ischemic heart disease. Because of the potential detrimental consequences of B2R antagonism, especially in the cardiovascular system, the B2R has become less attractive as a drug target over the past decade. On the other hand, the B1R is induced by certain inflammatory mediators. Its activity persists in damaged or inflamed tissues by promotion of neutrophil accumulation and by the further release of inflammatory cytokines, such as interleukins (IL-6, IL-1β) and tumor necrosis factor-α, which sustain a positive feedback loop between B1R expression and inflammation.[22] The disease-dependent expression pattern of the B1R and its absence in normal tissues may minimize the incidence of side effects of B1R antagonism. Therefore, in recent years research efforts in the pharmaceutical industry and academia have been focused on the discovery of B1R antagonists for the treatment of pain and inflammation-related diseases.

The roles of BK receptors in pain and inflammation have been established through studies of receptor KO mice as well as the effects of antagonists in animal pain models. The B1R-deficient mice are healthy, fertile, and normotensive, and they are hypoalgesic to noxious chemical and thermal stimuli under normal noninflamed conditions.[23] Under chronic inflammatory conditions, such as renal fibrosis, B1R KO mice have significantly lower expression of proinflammatory molecules, such as transforming growth factor-β, monocyte chemotactic protein-1, and IL-6.[24] Meanwhile, some anti-inflammatory mediators, such as IL-10 and heme oxygenase-1, are expressed at higher levels. As a result, certain inflammatory responses including thermal hyperalgesic responses to carrageenan or complete Freund's adjuvant (CFA) as well as lipopolysaccharide (LPS)-induced hypotension and carrageenan-induced pleurisy are absent or significantly reduced in B1R KO mice.[25] The B1R KO mice also show an attenuation of diabetic cardiomyopathy, with improved systolic and diastolic function in comparison with diabetic control mice, suggesting that the B1R is detrimental in diabetic cardiomyopathy, as it may mediate inflammatory and fibrotic processes.[26]

Several studies have demonstrated that the B1R may play a role in some aspects of neuropathic pain. For example, in the partial sciatic nerve ligation (PSNL) model of neuropathic pain, a non-peptide B1R antagonist was able to attenuate thermal hyperalgesia at day 21 but had no effect on tactile or cold allodynia.[27] Moreover, in the rat chronic constriction injury (CCI) model of mononeuropathy, elevated levels of B1R expression were associated with hyperalgesia after peripheral nerve injury, and the B1R antagonist [Leu^8]-des-Arg^9-BK (**5**, Figure 1) was antihyperalgesic.[28] The B1R is also implicated in the development of the hyperalgesia associated with diabetes. Thus, whereas B1R agonists significantly potentiated diabetes-induced hyperalgesia in mice, B1R antagonists completely reversed this hyperalgesia.[29] Similarly, B1R antagonists have been shown to be antihyperalgesic in a rat model of chemotherapy-induced neuropathy.[30] The interruption of pain signaling by B1R antagonists may be the result of blockade of B1R expressed on nerve terminals in the dorsal horn or on intrinsic spinal cord neurons.[31]

The discovery of constitutive B1R expression in the rat nervous system inspired an investigation of the role of neuronal B1R in inflammatory hyperalgesia.[32] Twenty-four hours after injection of CFA into one hind paw, there was a significant increase in B1R protein expression in both ipsilateral and

contralateral dorsal root ganglion neurons. The B1R agonist **3** caused a marked mechanical hyperalgesia of the contralateral, uninflamed hind paw by either intraplantar or intrathecal administration, correlating with the observed contralateral and ipsilateral increases in receptor levels. In behavioral experiments, the B1R antagonist des-Arg^{10}-HOE140 (**6**), administered by either intrathecal or systemic routes, attenuated CFA-induced mechanical hyperalgesia in the inflamed paw but did not ameliorate mechanical allodynia. Together, the immunolocalization of B1R to sensory neurons, their up-regulation following inflammation, and the pronounced behavioral effects of intrathecally administered B1R agonist **3** suggest that centrally expressed B1R is indeed functional. Centrally expressed B1R is therefore likely to play a more extensive role in hyperalgesia than previously thought.

B1R Antagonist Discovery Tools. B1R antagonists may be identified using receptor binding assays, wherein membrane preparations of MRC5 human fibroblasts and transfected HEK 293 cells expressing human (or other species) B1R are most commonly used.[33] Functional antagonist potency is assessed in calcium flux assays, using calcium-sensitive dyes and fluorescence imaging plate readers (FLIPR) or using the calcium-sensitive protein aequorin and luminometers. Inhibitory constants (IC_{50} and K_i) have been determined radiometrically using radioligands and cells stably expressing B1 receptors, such as Chinese hamster ovary (CHO) cells.

Preclinical animal models of inflammatory and neuropathic pain have been used to evaluate the in vivo efficacy of B1R antagonists. Two commonly used inflammatory pain models entail carrageenan-induced acute hyperalgesia and CFA-induced persistent hyperalgesia.[34] Interplantar injection of carrageenan or CFA in the hind paw of a Sprague−Dawley rat induces hyperalgesia, which is suppressed in a dose-dependent fashion by administration of a B1R antagonist. The effect of B1R antagonists on acute nociceptive responses in rat is also evaluated in the PSNL and CCI models of neuropathic pain, where the latency to withdraw the hind paw from either mechanical or thermal stimuli is measured after administration of a B1R antagonist to the injured rat.[28]

The lack of receptor homology across species has been a key hurdle facing the development of B1R antagonists. As the rodent B1R is only 71% homologous to its human orthologue,[35] it is not unusual to identify potent antagonists of the human B1R (hB1R) that are not active in rodent B1R assays.[36,37] Such compounds cannot be evaluated in the many existing preclinical rodent models of pain and inflammation. Conversely, rodent specific compounds would not be useful as human therapeutics. To circumvent this obstacle, transgenic rats or mice have been generated and characterized, in which the hB1R is constitutively overexpressed.[38] In addition, most potent hB1R antagonists are comparably active against rabbit B1R likely because of the higher homology (82%) between human and rabbit B1R. Therefore, pharmacodynamic (PD) and efficacy models have been developed in rabbits to evaluate the effectiveness of B1R antagonists in vivo.[39] In a PD model, for example, functional B1R in rabbit vasculature is induced by intravenous (iv) administration of LPS, which leads to a decrease in rabbit blood pressure upon treatment with the B1 agonist **3**. This hypotensive response is reversed in a dose-dependent manner by pretreatment with ascending doses of B1R antagonists prior to agonist treatment. Further, rhesus monkeys have been used in similar B1R agonist-induced hypotension models.[40] Rabbits have also been used in an antinociceptive model, wherein mechanical hyperalgesia was induced in the rabbit hind paws using CFA.[41] In this study, a peptide B1R antagonist (administered iv) dose-dependently inhibited the spinal nociceptive reflex response to a noxious pinch of the inflamed rabbit hind paw.

Development of B1R Antagonists as Analgesic Agents. Animal studies performed over the past decade suggest that the B1R plays an essential physiological role in the initiation of inflammatory responses as well as the development of neuropathic pain associated with spinal nerve ligation, and B1R antagonists may be therapeutically useful as analgesic agents. Currently, μ-opioids are the most common choice for chronic pain management. Although they provide adequate pain relief in many patients, their major disadvantage stems from their side effect profile, which includes constipation, nausea, vomiting, and respiratory depression. An advantage of B1R antagonists over opioids may be the absence of sedation and dependence and a lack of disruption of sensorimotor function. Effectiveness in animal models of neuropathic pain, sometimes in the absence of inflammation, also distinguishes B1R antagonists from the other widely used analgesic class, the nonsteroidal anti-inflammatory drugs (NSAIDs).

Although there is abundant evidence of the prominent role of bradykinins and their receptors in pain and inflammatory diseases, only a limited number of human clinical studies have thus far been conducted in the areas of pain,[42] asthma,[43] rhinitis,[44] and septic shock,[45] which have demonstrated some degree of efficacy with BK antagonists. From these efforts, only one compound, icatibant (**7**, Figure 1), a selective B2R antagonist, reached the market in 2008 for the treatment of hereditary angioedema (HAE). It is a hydrophilic decapeptide and must be administered parenterally. Like **7**, many early B1R/B2R antagonists are peptide analogues of the BK receptor agonists, **1**−**4**.[46] They were generated by replacement of the aromatic amino acids in endogenous BK agonist peptide fragments with aliphatic ones or by substituting natural amino acids with unnatural ones (Figure 1). These peptide analogues of **1** have been fundamental in developing an understanding of the roles of kinins and their receptors in physiology and pathophysiology. Despite the effectiveness of these peptide-based B1R/B2R antagonists in animal models, their peptidic nature has precluded their further development as oral agents. Significant effort has been directed toward discovery and optimization of novel, orally active, and well tolerated small molecule B1R antagonists, which is the focus of this Perspective. Preclinical data with a number of these small molecule B1R antagonists supports the notion that targeting B1R brings new therapeutic opportunities for the treatment of pain and inflammation.

Selective Small Molecule B1R Antagonists

More than a decade after the discovery of the first small molecule B1R antagonist in 1997, a large number of B1R antagonists have been reported in the primary and patent literature. The majority fall into two chemical classes: arylsulfonamides and amides. The first class of compounds features a hydrophobic group connected to a basic group through a sulfonamide or a sulfone linker. The second class of B1R antagonists contains a biphenylbenzylamine group as the amine part of the amide.

Sulfonamide-Based B1R Antagonists. Nearly 2 decades after the discovery of peptidic B1R antagonists, the first

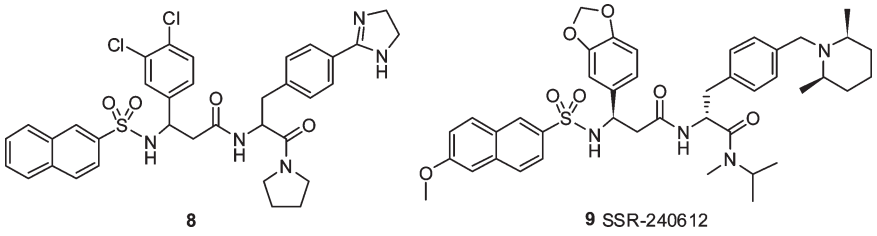


Figure 2. First generation sulfonamide B1R antagonists.

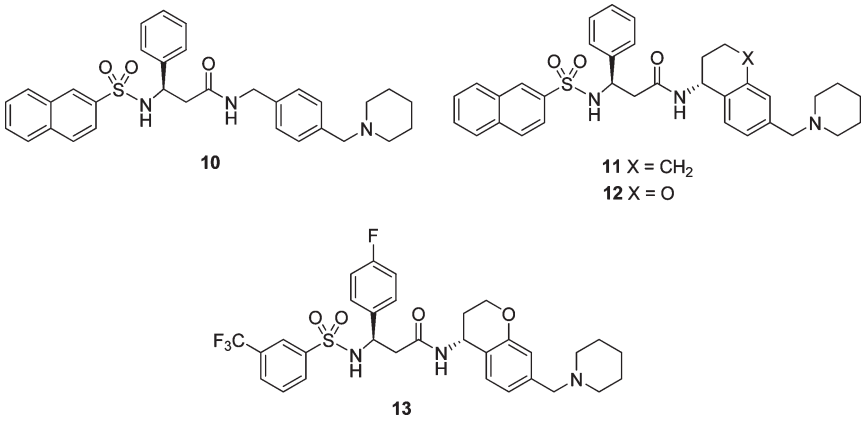


Figure 3. β-Phenylglycine sulfonamide B1R antagonists.

small molecule antagonist was disclosed in a patent application in 1997 (Figure 2).[47] This chemical series, exemplified by **8**, was a dipeptidomimetic consisting of one *N*-(arylsulfonyl)-β-amino acid and one phenylalanine amide. One of the lead compounds, **9** (SSR-240612), was a potent hB1R antagonist (K_i = 0.48−0.73 nM) in in vitro binding and functional assays in several cell lines and was more than 500-fold less potent at B2R.[33] In vivo, **9** was orally active in a number of models of neurogenic inflammation and inflammatory pain. For instance, **9** inhibited B1R agonist-induced paw edema in mice (3 and 10 mg/kg po, and 0.3 and 1 mg/kg ip) and reduced capsaicin-induced ear edema in mice (0.3, 3, and 30 mg/kg po). The compound also inhibited UV-induced thermal hyperalgesia in rat paw at oral doses of 1 and 3 mg/kg. It prevented neuropathic thermal hyperalgesia in the rat CCI model at 20 and 30 mg/kg po. Despite its impressive efficacy in animal models, the development of **9** was discontinued for undisclosed reasons after a phase II clinical trial. Nevertheless, it continues to serve as a tool for further exploration of the pathophysiological role of the B1R. For example, topical application of **9** has since been found to cause a significant inhibition of the capsaicin-induced cutaneous neurogenic inflammatory response in mice, suggesting that B1R antagonists may be useful in the treatment of some cutaneous inflammatory diseases.[48]

Disadvantages of **9** as an oral agent are its high molecular weight (743 Da) and peptidic nature (two amide bonds). By elimination of one of the amide bonds in **9**, β-phenylglycine sulfonamide **10** (Figure 3) was identified as a moderately active B1R antagonist (IC_{50} = 447 nM).[49] As part of a strategy to decrease conformational entropy, the benzylamide of **10** was constrained with a six-membered ring. The resulting tetralin **11** was ~2000-fold more potent than the acyclic **10**. The (*R*)-configuration was preferred at both the β-phenylalanine amino acid and the chiral center on the bicyclic amine. Compound **12**, the chroman analogue of **11**, was slightly more potent with regard to functional activity in hB1R-transfected CHO cells (Table 1). At a subcutaneous (sc) dose of 90 mg/kg, **12** was efficacious at suppressing plasma extravasation in two rodent models of inflammation (the rat pleurisy and the reverse passive Arthus models).

However, **12** was rapidly cleared and it was poorly bioavailable in rat (Table 1). A metabolite profiling study revealed that the naphthalene ring and the benzylic piperidine were the major sites of oxidative metabolism. Replacement of the left side naphthalene ring with a 3-trifluoromethylphenyl moiety led to **13**, which had similar potency as **12** but with an improved half-life ($t_{1/2}$), clearance rate (Cl), and oral bioavailability (*F*), even though the benzylic piperidine ring was unmodified.[50] The introduction of a 4-fluoro substituent in the central phenyl ring of **13** was also beneficial for its functional potency. Compound **13** was potent against human (IC_{50} = 0.8 nM) and rabbit (IC_{50} = 10.4 nM) B1R but was less active against rat B1R (IC_{50} = 127 nM). Therefore, instead of using traditional rodent models of inflammation and pain, two rabbit models were established to evaluate the in vivo activity of **13**. In the rabbit blood pressure model, sc administration of **13** significantly reduced the B1R agonist-induced hypotension in a plasma concentration-dependent manner, exhibiting a maximum decrease of 47% in the hypotensive response at the highest plasma concentration (~ 4 μM). In an inflammatory pain model, mechanical hyperalgesia was introduced in the rabbit hind paw using local injection of carrageenan. At a dose of 10 mg/kg sc, **13** caused a 45% reversal of hyperalgesia compared with vehicle.

The β-phenylglycine moiety of sulfonamide **13** may also be replaced by a piperidine 2-acetyl group, as in **14** (Figure 4).[51] Compound **14** was a potent antagonist of hB1R (IC_{50} = 13 nM) and demonstrated an oral bioavailability of 19% in the rat (Table 1). In this piperidine sulfonamide series, a change from tetralin to chroman on the right-hand ring system resulted in approximately 7-fold loss of potency.

Table 1. Binding and Functional Affinities of B1R Antagonists and Rat PK Profile of Selected Compounds

		IC_{50} (nM)				
compd	hB1 binding,[a] K_i (nM)	hB1 functional affinity[a]	other species, functional affinity[a]	$t_{1/2}$[b] (h)	Cl[b] ((mL/min)/kg)	F[c] (%)
10	132	447				
11	0.24	5.5				
12	0.77	3.4	17 (rabbit) 4 (rat) 0.4 (AGM)	1.4[d]	84.1[d]	4[e]
13	0.4	0.8	10.4 (rabbit) 127 (rat)	2.5[d]	24.8[d]	13
14	23	13		2[d]	36.7[d]	19
17	4.1	4.3	11.2 (rabbit)	4.7[f]	121[f]	18[g]
18	0.034	0.18	0.26 (rabbit) 16.3 (dog) 163.5 (rat)			
19	0.19	0.14				
20		0.8				
21	67					
22	300					
23	11	37[h]				
24	0.35		6.5 (mouse)[h]			
28	45			0.3	26	3
29	0.25			14	29	41
30	0.7			9.7	43	3
32	8−14	33	7.7 (monkey)[h]	2.7i		42[j]
33	0.59	1.9	2.8 (rat)	2.7		3.4
35	200					
36	1.2	1.4		0.6	42	21
37	0.4	0.25	0.69 (rabbit)[h] 35.3 (rat)[h]	2.1	11	50
38	0.5	1.17	3 (rabbit)[h] 119 (rat)[h]	7.8	9	73
39	13					
40	0.83			1	29	5
41	2			1.2	17	18
42	0.6	0.45		1[k]	16[k]	
43	815					
44	0.045					
45	11.8			0.15	35	9
46	52000					
47	3450			6.6	4.2	35
48	63			9.5	9.3	26
49	0.13			1.44	25	14

[a] Values reported are an average of at least three determinations. [b] Sprague−Dawley rats ($n = 3$) dosed at 2 mg/kg iv. [c] Dosed at 10 mg/kg po. [d] Dosed at 1 mg/kg iv, formulated in 100% DMSO. [e] Dosed at 3 mg/kg po, formulated in 1% Tween-80, 2% HPMC in water. [f] Dosed at 3 mg/kg iv in 100% water/methanesulfonic acid (pH 3.0). [g] Dosed at 3 mg/kg po in 100% water/methanesulfonic acid (pH 3.0). [h] Binding affinity, K_i value. [i] 0.7 mg/kg iv. [j] Dosed at 7 mg/kg po as a suspension in 0.5% methyl cellulose. [k] Cassette dose at 2 mg/kg iv.

Secondary amines with branched alkyl groups were preferred on the amine portion of this series.

An ether linker may be inserted between the piperidine sulfonamide and the tertiary amide. For example, **15** was reported to have an IC_{50} value of less than 10 nM in the calcium mobilization functional assay.[52] The arylsulfonamide moiety of **15** is connected with a basic tail through an ether amide linker. By use of the same 2,6-dimethyl-4-methoxyphenyl left-hand fragment as **15** and a phenylimidazoline group as the basic tail, **16** was active in in vitro binding and functional assays. In addition, **16** was efficacious in a mouse formalin-induced pain model,[53] with an ED_{50} value of 2.6 mg/kg iv.[54] The 2,6-dimethyl-4-methoxyphenyl moiety is also preferred in several other arylsulfonamide classes of B1R antagonists in the literature (vide infra).

Other more dramatic changes to the sulfonamide core of **13** involve replacement of the arylsulfonamide NH with CH_2 and further introduction of two hydroxyl groups to the backbone, resulting in a dihydroxylarylsulfone series, exemplified by **17** (Figure 5).[55] The replacement of the lipophilic core aryl group with the polar hydroxyl groups led to the largest benefit in attenuating metabolic instability of the previous sulfonamide series. Even though **17** did not show enhanced potency or an improved PK profile compared to **13** (Table 1), it was more efficacious than **13** in the rabbit hypotension model, with a plasma IC_{50} of 127 nM. In the rabbit carrageenan-induced mechanical hyperalgesia model, **17** reversed hyperalgesia with an ED_{50} of 20.2 mg/kg sc.

Another sulfonamide class of B1R antagonists contains the dihydroquinoxalinone (DHQ) ring system (Figure 6), identified through a high throughput screen (HTS).[37] Optimization of the initial hits, utilizing a homology model of the B1R based on the bovine rhodopsin crystal structure, led to **18**, which had picomolar affinity for hB1R (K_i = 0.034 nM).[56] The model suggests that **18** interacts mainly with the transmembrane (TM) helices TM1, TM3, TM6, and TM7 (Figure 7). The DHQ group is located deep within the crevice between TM3 and TM7, surrounded by hydrophobic residues Phe302, Ile113, and Trp103. The dichlorobenzene ring sits in another hydrophobic pocket surrounded by Ile97, Trp98, and Trp103 in TM1. The imidazoline ring is positioned to simultaneously interact with Glu273 and Asp291 in the extracellular loop between TM6 and TM7. The N1 of the DHQ acts as a hydrogen bond donor to the oxygen of the Asn114 side chain in the TM3 region. Residue Trp98 interacts with the sulfonamide oxygen via a hydrogen bond. The key interactions between the DHQ B1R antagonists and the receptor were also supported by site-directed mutagenesis studies. Compound **18** was highly selective for human and

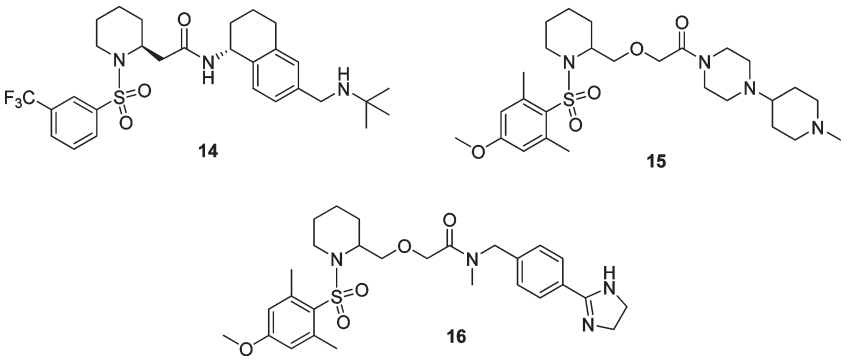


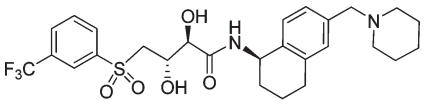
Figure 4. Piperidine sulfonamide B1R antagonists.

rabbit B1R over rat B1R (Table 1). In a rabbit model of inflammatory hyperalgesia, **18** inhibited the spinal nociceptive reflex response to a noxious pinch of an inflamed hind paw (induced with CFA) with an ED_{50} of 0.035 μg/kg when dosed iv. It was more effective than morphine (ED_{50} = 0.3 μg/kg) in the same model by dose, demonstrating the potent antinociceptive activity of **18**. Despite their impressive efficacy in animal pain models, **18** and related analogues do not possess acceptable PK properties, in particular oral bioavailability, to warrant further development.

The DHQ core provided superior potency as a replacement of the piperidine ring found in **14** and its analogues. These DHQs, exemplified by **19**, were very potent B1R antagonists (IC_{50} values of less than 0.2 nM) (Figure 6).[57] A molecular modeling study suggested similar binding modes for **19** in the B1R homology model as for **18**. The interaction of the endocyclic amide of the DHQ ring of **19** with Asn114 in the B1R binding pocket was thought to contribute to its improved potency relative to **13** and **14**. In addition, **19** exhibited greater metabolic stability and less plasma protein binding than **13**, making it a good starting point for further optimization.

It was disclosed in a patent application that the phenyl portion of the DHQ ring was not crucial for binding, as the nonaromatic 3-oxo-1,2,3,4-tetrahydropyrazine analogue **20** was also a potent hB1R antagonist (IC_{50} = 0.8 nM) in the functional assay.[58] Another variation on the DHQ core is the (1,2,3,4-tetrahydroisoquinolin-1-yl)acetic acid core, exemplified by **21** (Figure 6).[59] As the most potent compound in the series, **21** had a binding K_i of 67 nM. Although **21** has the same phenylimidazoline group as the highly potent DHQ **18**, it is about 2000-fold less active than **18**, probably because of the lack of the endocyclic amide of the DHQ ring. When the DHQ ring was opened, resulting in aspartic acid derivative **22**, potency also dropped significantly, emphasizing the crucial nature of the endocyclic amide's interaction with the receptor.[60]

From screening a 60000-member encoded combinatorial library, a reversed sulfonamide series of B1R antagonists was recently identified.[61] As represented by **23** (Figure 8), this chemical class differed from the previous series in two ways: the β-amino acid backbone was replaced with an α-amino acid, and the basic amine group was relocated from the amide side to the sulfonylaryl side. This novel series retained



17

Figure 5. Dihydroxylarylsulfone B1R antagonist.

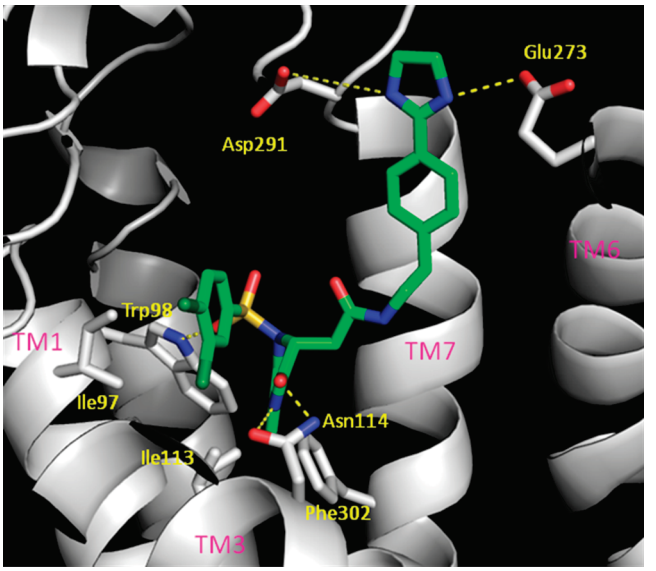


Figure 7. Homology model of B1R with **18** docked in the putative binding site.[56] Compound **18** is shown in green, and the binding site residues are colored in gray. Transmembrane domains and extracellular loops are represented in gray ribbons.

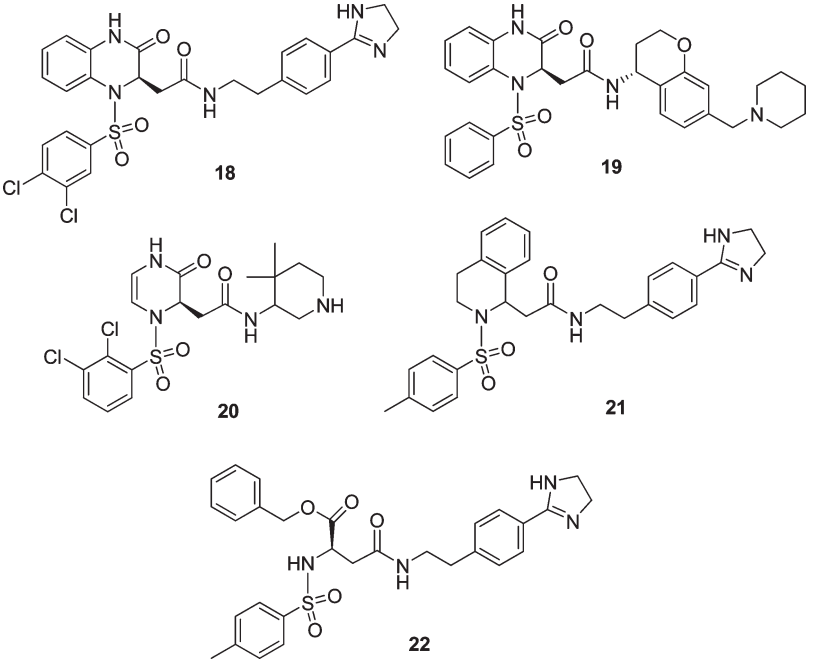


Figure 6. B1R antagonists containing a DHQ core or its variations.

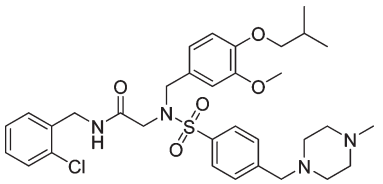


Figure 8. Reversed sulfonamide B1R antagonist.

potent B1R antagonist activity in both the hB1R binding (K_i = 11 nM for **23**) and functional (K_i = 37 nM for **23**) assays, and it may serve as a useful platform for the design of B1R antagonists in the future.

Most of the arylsulfonamide B1R antagonists previously discussed feature a β-amino acid backbone. There are also a number of non amino acid based sulfonamide analogues reported in the literature. For example, the sulfonamide ether **24** (LF22-0542) is an arylsulfonamide connected to a basic phenylimidazoline group via an ether and an acetamide linker (Figure 9). It was highly potent against both human (K_i = 0.35 nM) and mouse (K_i = 6.5 nM) B1R.[62] At a dose of 10 mg/kg sc, **24** elicited significant antinociceptive effects in the mouse acetic acid-induced abdominal constriction model of pain. Compound **24** also showed dose- and time-related inhibition of formalin-induced flinching in rats whether administrated by iv or sc routes. However, it was inactive when dosed orally at a dose of 30 mg/kg. In B1R KO mice, acetic acid and formalin responses were significantly reduced, whereas **24** had no additional effect on these animals, indicating that antagonist-mediated antinociception is B1R dependent. Compound **24** also alleviated thermal hyperalgesia in both acute (carrageenan-induced) and persistent (CFA-induced) inflammatory pain models in rats. In a mouse model of bone cancer pain, which involved the injection and confinement of 2472 sarcoma cells to the mouse femur, **24** (10 mg/kg sc, t.i.d.) was found to be effective in reducing pain-related behaviors at both early and advanced stages of bone cancer.[63]

As seen in arylsulfonamides **15**, **16**, and **24**, the hydrophobic 2,6-dimethyl-4-methoxyphenyl group was also preferred in several compounds (**25**−**27**) that have appeared in the patent literature (Figure 9). Using 1-cyclobutyl-4-pyrrolidin-1-ylpiperidine as the basic tail, **25** was equipotent to **24** with an hB1R K_i of 0.34 nM.[64] Compound **26**, featuring a bicyclic piperazine heteroaryl as the amide amine moiety, completely blocked CHO cell-expressed human and rat B1R at 10 μM in FLIPR assays.[65] By elimination of the ether linker in **24**−**26**, compound **27** was claimed as a BK receptor antagonist, but no biological data for these compounds were recorded in this patent.[66]

A recent series of sulfonamide B1R antagonists contains a 2-aminobenzophenone group (**28**−**30**, Figure 10).[67] Starting from HTS hit **28**, potency was improved dramatically by increasing chain length and appending a piperidine ring in the amide chain (**29**). Despite excellent binding affinity for hB1R and a good PK profile, **29** was susceptible to P-glycoprotein (P-gp) mediated efflux ((B/A)/(A/B) = 14) and was a potent hERG blocker (IC_{50} = 1 μM). P-glycoprotein, also known as MDR1, is found in capillary endothelial cells of the blood−brain barrier and is known to limit CNS penetration of certain compounds.[68] The basicity of the amine side chain was thought to be responsible for the undesired ancillary activities. Replacement of the urea linker with carbamate and transformation of the basic piperidine to

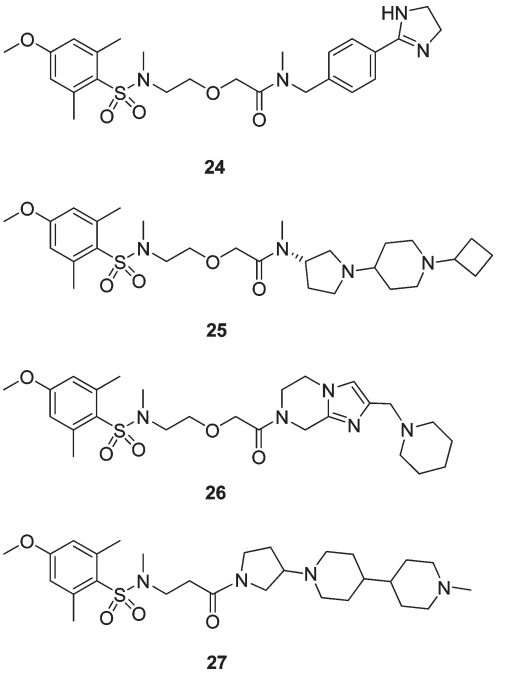


Figure 9. Compound **24** and other sulfonamide ether B1R antagonists.

a neutral tetrahydrofuran led to **30**, which was equipotent to **29**. Although **30** showed lower P-gp efflux and hERG activity, it had high clearance and poor bioavailability in rats (Table 1), in accordance with its poor liver microsomal stability. Nevertheless, **30** was examined in an ex vivo receptor occupancy study in transgenic rats.[69] At a dose of 12 mg/kg iv, **30** showed 74% and 80% receptor occupancy in brain and spinal cord, respectively, demonstrating its ability both to penetrate the blood−brain barrier and to possess high binding affinity for the B1R in the CNS. The ketone functionality bridging two phenyl groups in **28**−**30** can also be ether or thioether, according to a recently published patent application.[70] A representative compound, **31**, displayed an IC_{50} of less than 20 nM in the B1R functional assay.

Derived from a HTS hit, 2-alkylamino-5-sulfamoylbenzamide **32** (NVP-SAA164, Figure 10) exhibited high affinity for human and monkey B1R but no affinity for rat B1R (Table 1).[71,72] It possessed reasonable oral bioavailability of 42% and 35% in rat and dog, respectively. Oral administration of **32** produced a dose-related reversal of CFA-induced mechanical hyperalgesia and B1R agonist-induced hyperalgesia in hB1R transgenic mice but was inactive against inflammatory pain in wild-type mice, probably because of its lack of affinity for rodent B1R.

The sulfonamide based B1R antagonists share some common features: the requirements of an arylsulfonyl group, a hydrophobic group, and a basic side chain. This class of compounds is very potent for B1R and selective over B2R. They are efficacious in animal models of pain and inflammation, though in most cases, by parenteral administration, as a result of their suboptimal PK properties. The next genus of B1R antagonists to be discussed omits the sulfonamide functionality but maintains an amide motif linking a hydrophobic group and a basic moiety.

Amide-Based B1R Antagonists. Developed at the same time as the DHQ sulfonamide series, the benzodiazepines

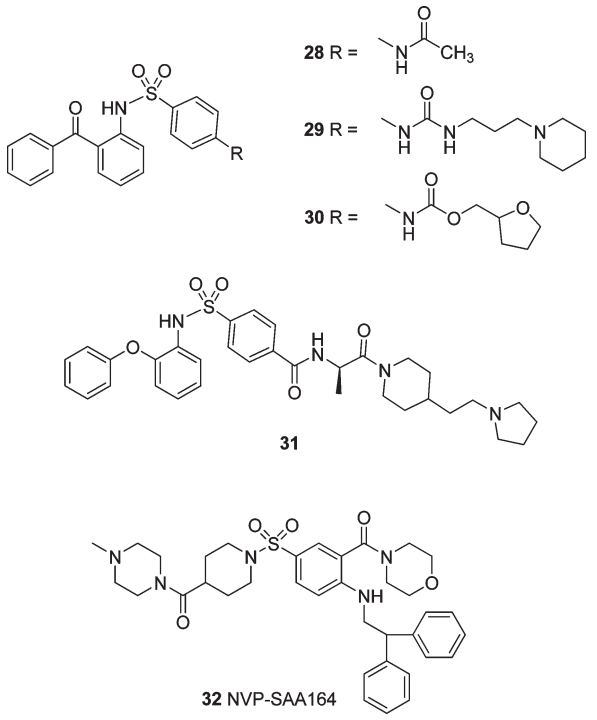


Figure 10. Miscellaneous sulfonamide B1R antagonists.

were also identified from a HTS (Figure 11). The most potent benzodiazepine, **33**, possessed subnanomolar hB1R binding affinity (K_i = 0.59 nM).[73] It was also potent for rat B1R (K_i = 0.92 nM). At 3 mg/kg ip, **33** showed similar efficacy to morphine with 89% suppression of the carrageenan-induced hyperalgesia in rats. However, the oral bioavailability of **33** in rats was only 3.4% (Table 1). Poor PK properties combined with some undesired off-target pharmacological activities led to the termination of this series.[74]

To improve the PK properties of the benzodiazepines, a search for a lower molecular weight surrogate as a replacement of the phenethylbenzodiazepine moiety of **33** was conducted, which led to the discovery of a novel series of benzimidazoles (Figure 11).[75] Potential alignments of the low-energy conformers of a benzodiazepine with the proposed benzimidazole template showed a significant overlap between these two structures with respect to several presumed key elements for receptor binding. In one of the benzimidazole analogues (**34**), 1-benzylbenzimidazole was connected through a β-alanine linker to the basic tail (2-imidazoline-5-aminopyridine). Compound **34** showed excellent antagonist potency at both cynomolgus and rat B1R with IC_{50} values of 2 and 0.8 nM, respectively. A number of benzimidazoles, including **34**, were assessed in MDR1-transfected MDCK cells and identified as P-gp substrates (efflux ratio of 42 for **34**). The basic tail of **34** was thought to facilitate the efflux by serving as a hydrogen bond acceptor.

The 2,3-diaminopyridine series of B1R antagonists (**35**−**38**, Figure 12) contains a privileged biphenyl structural motif found in several marketed drugs.[76] Compound **35** was a HTS hit with modest activity (K_i = 200 nM) toward hB1R.[39] Potency was greatly improved by the replacement of the *n*-pentanamide with a cyanoacetamide and by the incorporation of methyl groups at the benzylic carbon, the 4-pyridyl position and the 5-position of the distal aromatic ring (**36**).[77] However, **36** possessed undesirable PK properties, such as a short half-life ($t_{1/2}$ < 2 h) and a high clearance

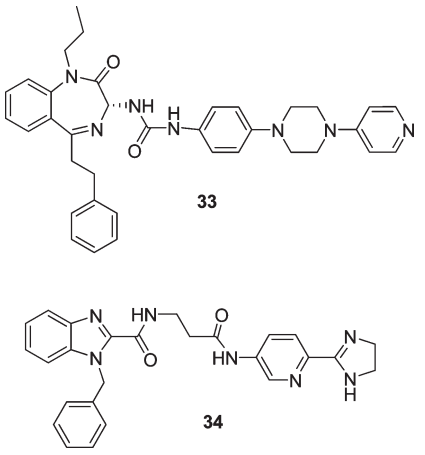


Figure 11. Benzodiazepine and 1-benzylimidazole B1R antagonists.

rate (Cl > 20 (mL/min)/kg) in rats (Table 1). Metabolic profiling of **36** revealed that the major metabolic liabilities were the oxidation of the 5′-methylaryl substituent and hydrolysis of the methyl ester. Substitution of the 5′-methyl group with a 3′-fluorine (**37**) resulted in increased potency and improved PK properties (Table 1). Ester isosteres, such as oxadiazoles, were prepared and found to have affinity for the hB1R comparable to that of the ester analogues but without acceptable PK properties. When N-2 substituted methyltetrazole was used as the ester isostere, **38** exhibited comparable potency to the methyl ester **37** and possessed improved PK properties. Both **37** and **38** were selective for human and rabbit B1R versus rat (Table 1). The compounds were potent with AD_{50} (dose required to block 50% of the agonist effect) values of less than 1 mg/kg iv in the rabbit blood pressure challenge model and were efficacious in the rabbit CFA-induced mechanical hyperalgesia model, with ED_{50} values of 2.1 and 3.1 mg/kg iv for **37** and **38**, respectively.

In an expanded SAR study, a number of diverse and potent analogues of **35** were identified (**39**−**42**, Figure 12).[78] For example, the proximal phenyl B-ring of **35** can be replaced by a piperidine (**39**), with retention of good receptor affinity (hB1R, K_i = 13 nM). Also alicyclic rings can be the alternatives for the distal phenyl C-ring of **35**. For instance, the *trans*-4,5-cyclohexene analogue (**40**) was about 10-fold more potent than its phenyl counterpart. However, the PK properties of **40** were unimpressive, probably because of the metabolically labile methyl ester moiety. Accordingly, the methyl ester was replaced with an isostere, 3-methyloxadiazole, providing **41** which demonstrated improved PK properties relative to **40** (Table 1). A further SAR study, which focused on B-ring modification, led to **42**, wherein the cyanoacetamide was changed to a 5-isoxazolamide, the B-ring was a 4-bis-substituted cyclohexane, and the methyl ester on the C-ring was converted to a trifluoromethyl group.[79] Compound **42** possessed good in vitro potency (K_i = 0.6 nM). In addition, **42** showed 77% and 84% of hB1R occupancy in brain and spinal cord, respectively, (with concentrations of 666 and 468 nM in brain and spinal cord) in an ex vivo receptor occupancy study in transgenic rats at a dose of 6 mg/kg iv. The ability of **42** to penetrate the blood−brain barrier and to occupy CNS B1 receptors was in concert with the finding that it was not a P-gp substrate (efflux ratio of 2.5, apparent permeability $P_{app} = 36 \times 10^{-6}$ cm/s).[80]

Despite excellent potency and reasonable PK properties, the diaminopyridine series, e.g., **37**, was found to be subject

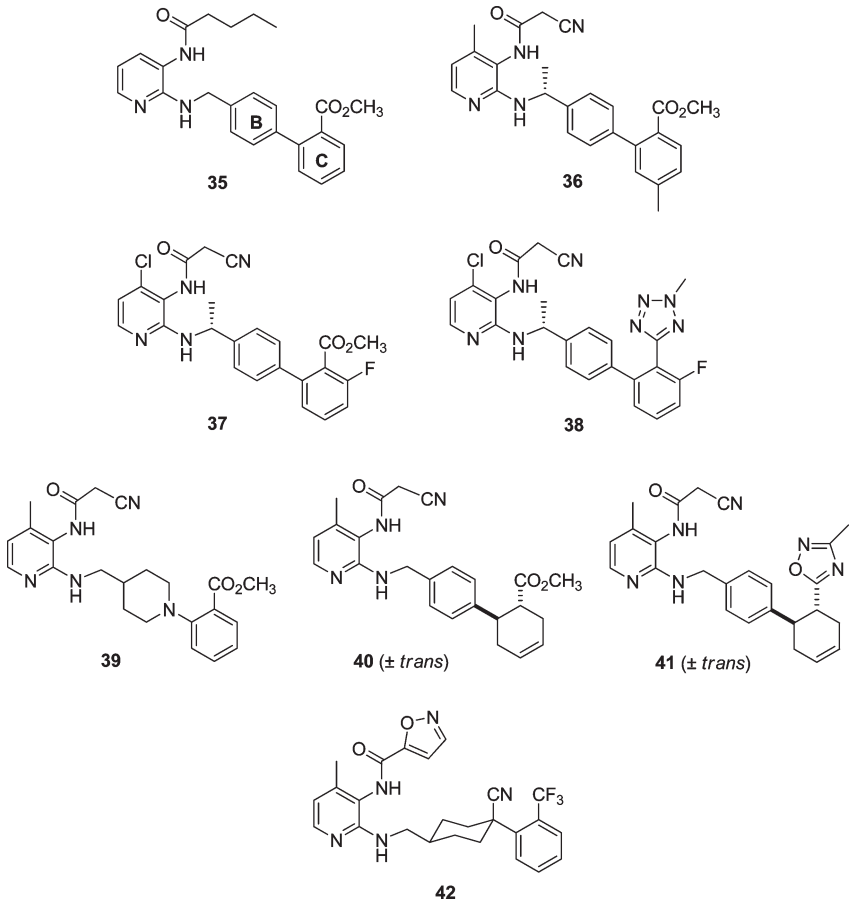


Figure 12. 2,3-Diaminopyridine B1R antagonists.

to bioactivation and conjugation to liver microsomal proteins and glutathione (Figure 13).[81] The electron-rich pyridine ring is prone to oxidation by cytochrome P450 3A, resulting in a reactive pyridine-2,3-diimine, which in turn is subject to nucleophilic attack to form a covalent conjugate. This reactive metabolite could haptenize protein or deplete glutathione, thus posing a risk for idiosyncratic drug reactions (IDR). As a result, development of this chemical class has been discontinued.

One approach to prevent the aforementioned IDR was to remove the 3-cyanoacetamide from the 2,3-diaminopyridine core. The truncated analogue (**43**) had modest hB1R binding affinity, with a K_i of 815 nM (Figure 14).[82] As the discovery of highly potent DHQ analogue **18** was facilitated by a theoretical study using a BR1-rhodopsin homology model, docking of **43** in that same model suggested that a basic pharmacophore extending from the 4- or 5-position of the pyridine ring might lead to compounds with enhanced affinity for hB1R. Accordingly, potency was dramatically increased when a 4-pyridylpiperazine carboxamide substituent was incorporated into the 5-position of the pyridine ring (**44**, K_i = 0.045 nM for hB1R). In this homology model, the biphenyl group of **44** occupies the same hydrophobic pocket that accommodates the DHQ moiety of **18**. Whereas the imidazoline moiety of **18** reaches Glu273 and Asp291, the 4-pyridylpiperazine tail of **44** is too extended and is positioned toward His199 in TM6. In contrast to compounds such as **37**, no glutathione adducts were detected when **44** was incubated with human and rat liver microsomes fortified with NADPH. However, this series was discontinued because of its suboptimal PK properties and undesirable affinity for P-gp.[74]

Another approach to overcoming the formation of reactive metabolites constituted the removal of the aminopyridine ring.[83] From the SAR data developed for the 2,3-diaminopyridine series, it seemed necessary to retain both N−H bonds, the *N*-acyl group, and the spatial orientation of the biphenyl motif in **45** (Figure 15). Although all of these elements were maintained while using a carbonyl group at the location of the pyridine nitrogen to maintain any relevant lone pair interaction, **46** did not retain the potency of **45**. The lack of conformational restriction in **46** may contribute to the loss of potency. To restrict the rotation around the carbonyl−C_α bond, a *gem*-dimethyl group was installed at the C_α position, resulting in **47**, which possessed weak hB1R binding affinity (K_i = 3.45 μM). Different cycloalkyl substitutions were examined at the C_α position to restore lipophilic interactions available to the aromatic pyridine ring of **45**. It was found that smaller rings were preferred, and ultimately, the cyclopropylcarbonyl moiety in **48** was found to be the best replacement for the diaminopyridine core, displaying a hB1R binding affinity (K_i = 63 nM) just 5-fold lower than **45**. Another attractive feature of **48** was the improved rat PK properties compared to **45** (Table 1), and it served as a lead for further optimization.

By application of the SAR learned from the previous series, two fluorine atoms were judiciously placed on the biphenyl rings to increase binding affinity, and a methyl group was incorporated at the benzylic carbon to mitigate the P-gp efflux activity of **48**. As a result, **49** had an excellent

hB1R binding affinity (K_i = 0.13 nM) and better P-gp transport properties (efflux ratio of 8.6 for **49** vs 18 for the corresponding *des*-methyl analogue).[40] To achieve a preferable P-gp efflux ratio of less than 3,[84] trifluoroacetamide was used to replace the trifluoropropionamide in **49**. Positioning of the fluorines α to the amide group makes the amide a poorer hydrogen bond acceptor and therefore less prone to recognition by P-gp. This hypothesis was fully realized in **50**, which had a P-gp efflux ratio of 2.3, in accordance with the finding that **50** had an Occ_{90} (the concentration required to occupy 90% of CNS hB1R) of 440 nM in transgenic rats overexpressing central hB1R. However, **50** exhibited poor oral bioavailability, coupled with short half-life and high clearance across multiple species (Table 2).

Modifications of the methyl ester produced compounds with higher P-gp efflux ratio and modestly improved oral bioavailability in rat. Replacement of 3′-fluorine at the distal aromatic ring of **50** with a chlorine (**51**, MK-0686) yielded a decrease in P-gp efflux (ratio of 1.9) without diminishing potency.[40] In addition, **51** exhibited significantly improved PK properties in both dog and rhesus monkey compared with **50** (Table 2). Even though the PK properties in rat were still suboptimal, **51** demonstrated superior stability in human microsomes and hepatocytes relative to rat, dog, and rhesus monkey, indicating that it may have adequate bioavailability in humans. Therefore, **51** was selected as a clinical candidate.

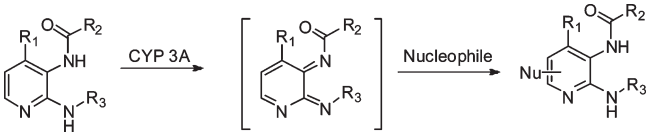
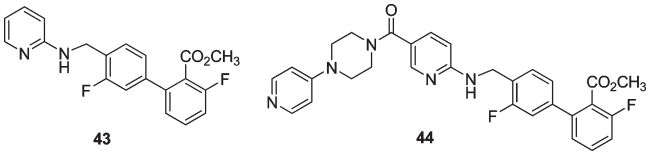


Figure 13. Bioactivation and conjugation of 2,3-diaminopyridines.

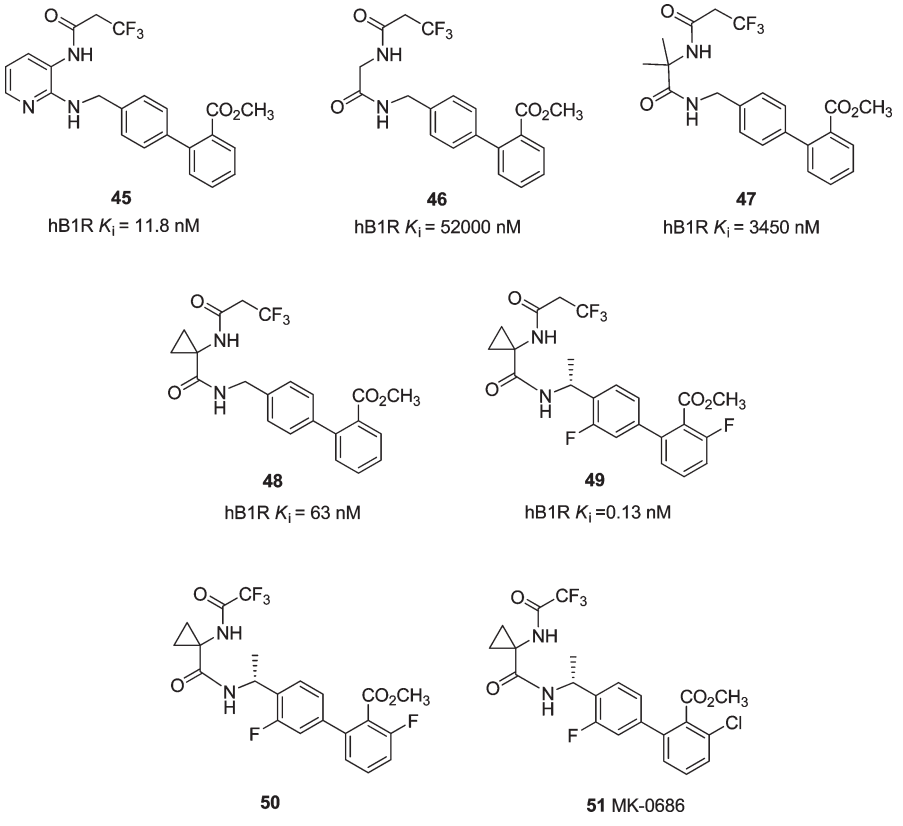


43 **44**

Figure 14. 5-Piperazinylpyridine carboxamide B1R antagonists.

As **51** was active against rhesus monkey B1R (K_i = 2.0 nM), it was examined in a rhesus monkey blood pressure model, wherein hypotension was induced by the B1R agonist **3**. Compound **51** dose-dependently reduced the hypotensive effects, with an AD_{90} (dose required to block 90% of the agonist effect) of 47 μg/kg iv.[40] In the previously described transgenic rat model, **51** exhibited an Occ_{90} of 520 nM (iv) in the rat CNS, a reasonable brain level that may be attainable in humans based on its low human P-gp substrate activity. As a poor rodent B1R antagonist (rat B1R K_i = 1646 nM), **51** is not suitable for evaluation in rodent pain models. However, its efficacy was determined in a CFA-induced mechanical hyperalgesia model using a humanized B1R knock-in mouse,[85] wherein oral administration of **51** dose-dependently reversed the hyperalgesia with an ED_{50} value of 9.8 mg/kg. At the maximum dose of 60 mg/kg, it produced similar efficacy (100%) as naproxen in this model. It is noteworthy that **51** did not affect CFA-induced hyperalgesia in the wild-type mice.

In a 4-week safety assessment study in rats, **51** was administered orally at doses of 30, 60, and 150 (mg/kg)/day. Although its safety profile was acceptable, the C_{max} and AUC of **51** decreased 2- to 4-fold during the first several days of dosing, indicating rapid clearance of the compound in



45 hB1R K_i = 11.8 nM

46 hB1R K_i = 52000 nM

47 hB1R K_i = 3450 nM

48 hB1R K_i = 63 nM

49 hB1R K_i =0.13 nM

50

51 MK-0686

Figure 15. Cyclopropane carboxamide B1R antagonists. Discovery of the clinical candidate **51**.

Table 2. Binding Affinities, Physical Properties and PK Profiles of Selected B1R Antagonists

			rat PK[c]			dog PK[d]			monkey PK[e]						
compd	hB1 K_i (nM)[a]	P-gp[b]	F (%)	$t_{1/2}$ (h)	Cl ((mL/min)/kg)	F (%)	$t_{1/2}$ (h)	Cl ((mL/min)/kg)	F (%)	$t_{1/2}$ (h)	Cl ((mL/min)/kg)	Occ_{90}[f] (nM)	AGM B/P[g]	PB[h] (%)	log P
50	0.57	2.3	21	0.5	42	5	1.7	21	2	2.2	28	440			
51	0.44	1.9	34	0.4	40	33	1.8	9	31	1.7	13	520	0.4	98.6	> 3.6
52	0.77	1.9	21	1.2	19.6							350	0.5	97.2	3.2
53	2.6	2.2	19	1.3	16.6	66	6.6	6.2	12	2.5	16.9	210	0.8	97.5	3.3
54	0.4	5.1													
55	0.5	2.1	44	4.5	2.4	94	21	1.2	93	6.2	1.6	540	0.7		
58	59														
59	24	1.6					1.4[i]	16[i]							
60	0.35	1.6	47	5.6	7.8		3.6[i]	3.2[i]							
61	0.66	1.6	34	2.0	4.9	84	11	0.45	52	1.3	7.1	140	1.4		
	4.47[j]														
63	0.41	2.0	62	5.6	2.2	91	20.3	1.0				140	0.63		
65	0.26		57[k]	1.7[l]	16[l]				100[m]	3.9[n]	8.2[n]				

[a] Values represent the numerical average of at least two experiments. Interassay variability was ±25%. [b] MDR1 directional transport ratio (B to A)/(A to B). Values represent the average of at least three experiments. Interassay variability was ±20%. [c] Sprague–Dawley rats (n = 3): 10 mg/kg po; 2 mg/kg iv. Interanimal variability was less than 20%. [d] Mongrel dogs (n = 2): 3 mg/kg po; 1 mg/kg iv. Interanimal variability was less than 20%. [e] Rhesus monkeys (n = 2): 3 mg/kg po; 1 mg/kg iv. Interanimal variability was less than 20%. [f] The concentration required to occupy 90% of CNS hB1R. Values are the mean of at least eight experiments. [g] CNS penetration level (brain/plasma ratio) in African green monkey. Values are the mean of two experiments. [h] Protein binding measured using 10% rat serum. [i] Beagle dogs (n = 2): 0.25 mg/kg iv, as a cocktail of three to six compounds (plus reference standard) in DMSO. Interanimal variability was less than 20%. [j] hB1R functional assay data [FLIPR, IC_{50} (nM)]. [k] Dosed at 10 mg/kg po in 2% Tween-80/saline. [l] 2.5 mg/kg iv in 2% Tween-80/saline. [m] Dosed at 5 mg/kg po in 10% Solutol. [n] 1 mg/kg iv in 10% Solutol.

rats.[74] Similarly, after 4 weeks of treatment of **51** (q.d., po), rhesus monkeys exhibited significantly reduced systemic exposure of the compound in a dose-dependent manner, which was attributed to an autoinduction mechanism through CYP2C75, as **51** was found to be both a substrate and an inducer of CYP2C75.[86] It was later found that **51** was an activator of the pregnane X receptor (PXR), an activity that is commonly found with trifluoroacetamide-containing compounds.[87] Activation of PXR leads to the induction of the cytochrome P450 isozyme 3A4, which is responsible for the metabolism of approximately 60% of marketed drugs.[88] Thus, PXR activation caused by **51** may raise the risk of drug–drug interactions. However, **51** had a half-life of 9–10 h in human, and there were no observed decreases in C_{max} over a 4-week period of human dosing despite the PXR activation observed in the safety studies. Compound **51** has been in phase II clinical trials for the treatment of osteoarthritis and postherpetic neuralgia; however, no recent information on efficacy for either indication has been reported.

In an effort to mitigate the high lipophilicity and low aqueous solubility of **51**, a nitrogen was inserted into the proximal phenyl ring to provide **52** (Figure 16).[89] Pyridine **52** showed similar potency with an improved half-life and reduced clearance in the rat (Table 2). Addition of the 5′-chloro (**53**) on the distal phenyl ring of **52** did not improve PK properties in rat or in rhesus monkey, but a significant increase in bioavailability (66%) and half-life (6.6 h) was observed in dog. Both **52** and **53** were not P-gp substrates, with transport ratios of less than 2.5. Although **53** was less potent in the hB1R binding assays (K_i = 2.6 nM vs 0.77 nM for **52**), it exhibited the best CNS B1R occupancy (Occ_{90} = 210 nM) in the series as well as a good brain to plasma ratio (B/P = 0.8) in African green monkey (AGM). The subtle improvement in physical properties (lower log P and protein binding) of the pyridine analogues may contribute to their improved PK properties, CNS receptor occupancy, and brain penetration in the monkey (Table 2). However, these pyridine-containing compounds still possess the PXR activation liability.

To mitigate the PXR activation associated with the trifluoroacetamide appendage of **51**, a variety of heterocyclic replacements were evaluated, among which the isoxazoles were most promising.[90] 3-Methoxyisoxazole analogue **54** (Figure 16) maintained potent hB1R binding affinity (K_i = 0.4 nM) and displayed 50% less potential to activate PXR compared to **53**. However, it was still a P-gp substrate. When a difluoroethyl ether group was used to replace the metabolically labile methyl ester, **55** exhibited a desirable P-gp profile (ratio of 2.0) and the lowest PXR activation potential among the series (8% at 10 μM, relative to rifampicin). Moreover, **55** was very potent at hB1R (K_i = 0.5 nM) and had excellent PK properties in three species as well as high brain exposure in AGM (B/P = 0.7) (Table 2). Therefore, **55** was selected for further evaluation as a backup to the clinical candidate **51**.

In two recently published patents, pyrimidine-5-carboxylic acid amide was used as the replacement of trifluoroacetamide moiety in **51** (Figure 17). Exemplified by **56**, this chemical class substitutes the biphenylbenzylamine motif in the other cyclopropane carboxamide series (**48**–**55**) with a pyrrolidinecarbonylbenzylamine group.[91] Most preferred compounds exhibited IC_{50} values of less than 50 nM in the hB1R functional assay. No biological data were revealed for the other series, biphenyl ether **57**.[92]

An alternative approach to reduce the potential of PXR activation by **51** was to truncate the offending amide group and to replace it with a hydroxyl group to maintain the necessary hydrogen bond of the amide N–H. The resulting 1-hydroxycyclopropylamide **58** displayed only a 40-fold decrease of the hB1R binding affinity relative to its trifluoroacetamide counterpart (Figure 18).[93] However, **58** was susceptible to CYP-mediated oxidative opening of the strained cyclopropane ring to form undesired, reactive metabolites. Simple opening of the cyclopropyl ring led to a *gem*-dimethyl compound (not shown), which exhibited only a 2-fold decrease of the hB1R binding affinity compared with **58**. Replacement of one of the methyl groups with a trifluoromethyl moiety electronically affected the adjacent hydroxyl

group, which proved to be beneficial for the lipophilic interactions between the receptor and this portion of the molecule. The (*R*)-enantiomer **59** had a K_i of 24 nM, while its (*S*)-enantiomer was 24-fold less potent. On the basis of the SAR developed in the previous cyclopropane carboxamide series, a benzylic methyl, a central pyridine ring, and a chlorine at the 5′-position of the distal phenyl ring were incorporated into the molecule (**60**), which resulted in a 70-fold increase in potency (Table 2). Although **60** had reasonable PK properties in rat, it showed a relatively short half-life and moderate clearance in dog. Additional utilization of the 5-methyloxadiazole as an ester surrogate led to **61**, which exhibited excellent activity in binding and functional assays, a good P-gp efflux ratio (1.6), better CNS exposure in AGM (B/P = 1.4), and desirable PK parameters across three species (Table 2). More importantly, it occupied 90% of the hB1R expressed in rat CNS at 140 nM, one of the lowest Occ_{90} values determined in the series. In the previously described rhesus monkey blood pressure model, **61** dose-dependently reversed the hypotensive effect, with an AD_{90} of 17 μg/kg iv. Taken together, these optimized properties support the selection of **61** as the third generation backup compound to the clinical candidate **51** for the treatment of inflammatory pain.

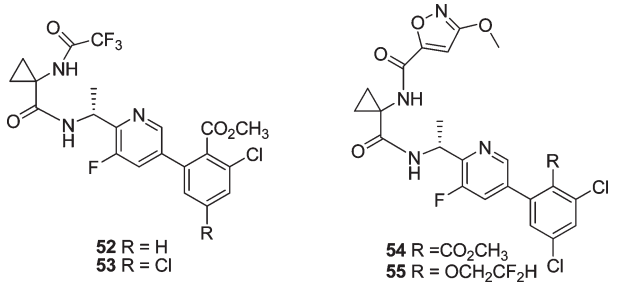


Figure 16. Optimization of **51** led to the backup clinical candidate **55**.

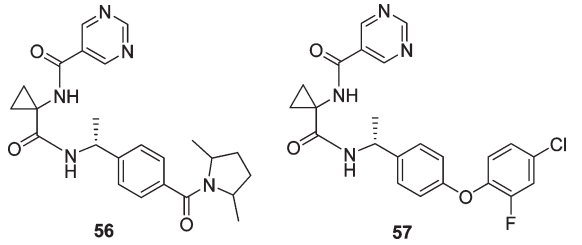


Figure 17. Pyrimidine-5-carboxylic acid amide B1R antagonists.

Later, **61** was found to be metabolized exclusively by CYP2B6, which resulted in a major circulating metabolite, **62** (Figure 18).[94] This metabolite was also a potent hB1R antagonist and showed good PK properties with a long $t_{1/2}$. Efforts were then directed toward preparation of compounds that would be oxidized by multiple CYP enzymes and/or have alternative clearance pathways to avoid the production of a major circulating active metabolite. Since the 5-methyloxadiazole of **61** was the major site of metabolism, it was replaced with a *N*-2-methyltetrazole, which had been previously found to be a good ester isostere in the diaminopyrine analogue **38**. Oxidative demethylation of the *N*-2-methyltetrazole by CYP2B6 led to the acidic tetrazole, which had no significant affinity for hB1R. To introduce additional oxidation potential to the molecule, an aliphatic cyclohexane ring was employed to replace the metabolically resistant trifluoromethyl group. As a result, **63** exhibited excellent binding affinity, low P-gp transport activity, and superior PK properties in rat and dog (Table 2). Although **63** had a lower brain to plasma ratio (0.63) in AGM relative to **61**, it was equally potent in the ex vivo receptor binding assay in transgenic rats, with an Occ_{90} of 140 nM. Metabolic profiling revealed that **63** was metabolized by multiple pathways in human microsomes and hepatocytes, and no major active circulating metabolites were observed. Accordingly, **63** was selected for preclinical development as a backup to **61**.

As one of the first small molecule B1R antagonists disclosed in the literature, **64** (PS020990) was identified from the screening of an encoded combinatorial chemistry library.[95] It was a potent hB1R antagonist (K_i = 6 nM) and inactive at B2R. Furthermore, it was more than 1000-fold specific for B1R vs a variety of other receptors, ion channels, and enzymes. The structure of **64** was not initially disclosed. However, a subsequent patent application revealed the structure of **64** (Figure 19).[96] To date, no results have been published on the in vivo properties of **64**.

Compound **65** (ELN441958) is a novel B1R antagonist, which has been well characterized pharmacologically.[97] It is a spirocyclic benzamide with a basic pyridine (Figure 19). It exhibited high affinity for the hB1R (K_i = 0.26 nM) and was highly selective for B1R over B2R. In the in vitro functional FLIPR assay, **65** was selective for primate over rodent B1R, with a rank order potency of human ~ rhesus monkey > rat > mouse. Consistent with good permeability and metabolic stability in vitro, **65** showed moderate plasma half-lives, low clearance, and high oral bioavailability in both rat and rhesus

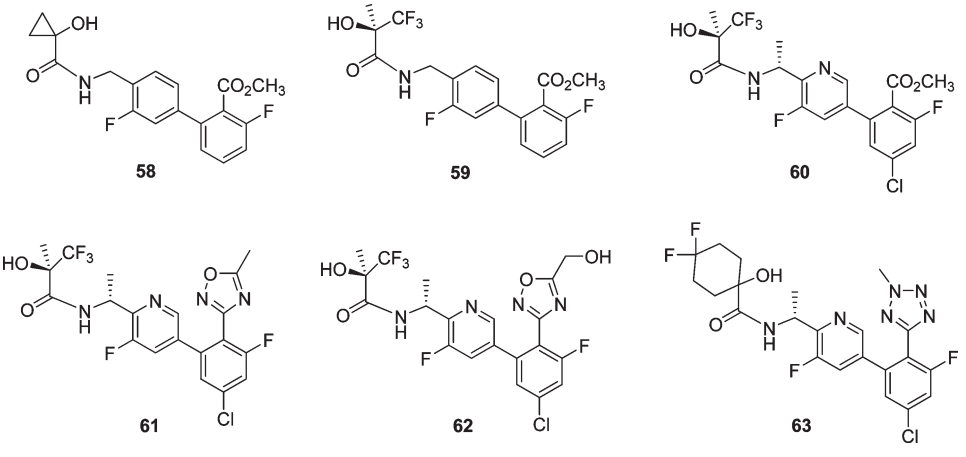


Figure 18. α-Hydroxyamide B1R antagonists.

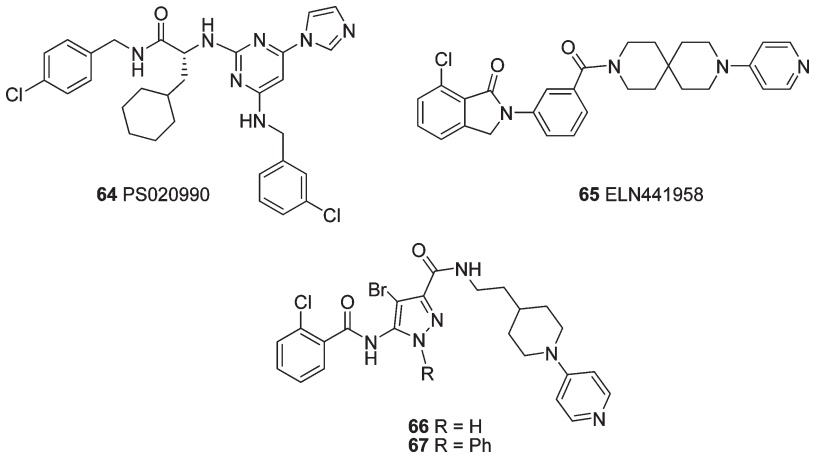


Figure 19. Miscellaneous amide B1R antagonists.

monkey (Table 2). However, **65** was found to be a P-gp substrate, which could limit its CNS exposure. Five minutes after an iv dose of 2.5 mg/kg, **65** achieved low micromolar brain levels in MDR1 KO mice but was below the limit of quantitation in wild-type mice, consistent with its P-gp-mediated extrusion from the CNS. Because **65** was more potent at primate than rodent B1R, it was evaluated in a primate pain model. In a dose-dependent manner, **65** reduced carrageenan-induced thermal hyperalgesia in a rhesus monkey tail withdrawal model, with an ED_{50} of approximately 3 mg/kg sc and with complete blockade at a dose of 10 mg/kg. In a rhesus monkey model of topical capsaicin-induced thermal allodynia, it also showed an antihyperalgesic effect at doses of 3−10 mg/kg sc. Given its low CNS exposure, **65** may act primarily at peripheral B1R.

The pyrazolecarboxamide series of B1R antagonists features a pyrazole with two appending amide bonds linking a hydrophobic phenyl ring and a basic tail, respectively. It is interesting to note that the lead compound **66** has the same 4-piperidylpyridine motif as **65** (Figure 19).[98] Although **66** was potent at hB1R in the functional FLIPR assay (IC_{50} = 3 nM), it was rapidly cleared in cynomolgus monkeys. In vitro assays of **66** and its analogues determined that N-glucuronidation of the pyrazole was the major clearance pathway in both monkey and human. Since this metabolic liability is known for the N-unsubstituted pyrazoles,[99] a series of N-substituted pyrazoles were prepared, among which *N*-phenyl substituted pyrazole (**67**) was almost equipotent to the unsubstituted pyrazole **66**, with an IC_{50} of 3.5 nM. As anticipated, **67** possessed higher metabolic stability because glucuronidation was blocked.

Conclusion

Many potent small molecule B1R antagonists have emerged from the sulfonamide and amide classes of compounds. Molecular modeling studies performed with compounds **18** and **44** suggest that these two structurally distinct chemical classes bind in the same region of B1R.[82] The hydrophobic binding pocket of B1R accommodates both the dihydroquinoxalinone moiety in the DHQ sulfonamide series and the biphenyl group in the amide series. Though not interacting with the same residues of the receptor binding site, the basic tails of these two series point toward the same hydrophilic region of the receptor. It is worth mentioning that the peptide-based B1R antagonists require a minimum of eight amino acids to achieve low nanomolar potency.[100] They may bind in different regions of the receptor from the small molecule antagonist binding site.

With the assistance of homology modeling, very potent B1R antagonists, such as **18** and **44**, were discovered. However, it was not potency but the physical profile and/or drug-like properties of the compounds that differentiated the clinical candidates from their analogues. Many compounds, including **18** and **44** in the above discussion, were abandoned because of poor oral PK properties, low CNS penetration, susceptibility for reactive metabolite formation, or lack of selectivity. Fortunately, compounds with improved properties, such as the clinical candidates **51**, **55**, **61**, and **63**, were identified through elegant medicinal chemistry efforts. Hopefully, molecular modeling studies, SAR data, and lessons learned from lead optimization will assist medicinal chemists in designing and developing new B1R antagonists with desirable physical and pharmacokinetic properties.

Although preclinical data of peptidic and nonpeptidic small molecule B1R antagonists suggest that the B1R is an attractive therapeutic target for both inflammatory and neuropathic pain, it requires further validation through human clinical studies. However, only a few compounds have come as far as phase II clinical trials, and neither **9** nor **51** has demonstrably progressed beyond this stage. The low success rate may be attributed to multiple factors, such as the species discrepancies in early animal studies, poor ADME properties of some compounds, or unexpected toxicity in clinical studies. Nevertheless, the recent launch of B2R antagonist **7** (icatibant, Firazyr) and the presence of another nonpeptidic B2R antagonist, anatibant, in advanced clinical trials create optimism for BK receptor antagonism as a therapeutic modality. It remains to be seen whether B1R antagonists, developed about 10 years later than B2R antagonists, will fulfill their promise as differentiated agents for the treatment of pain.

Acknowledgment. The authors thank Dr. Renee L. DesJarlais for molecular modeling and Dr. Christopher Flores for critical reading of the manuscript.

Biographies

Hui Huang received her B.Sc. in Physical Chemistry from Nanjing University, Nanjing, China, in 1993. She continued her graduate study in Bioorganic Chemistry at Northwestern University under the direction of Professor Richard B. Silverman. Her Ph.D. thesis work involved the design and synthesis of potent and selective inhibitors of nitric oxide synthase (NOS).

In 2000, Dr. Huang joined 3-Dimensional Pharmaceuticals (3DP) in Exton, PA, and worked on several lead generation projects. Since the acquisition of 3DP by Johnson & Johnson Pharmaceutical Research and Development, L.L.C., in 2003, she has been working on the lead optimization projects in the therapeutic areas of inflammation, metabolic diseases, and pain.

Mark R. Player received his undergraduate chemistry degree and his doctorate in Medicinal Chemistry from the University of South Carolina and the M.D. degree from the Medical University of South Carolina in 1986. His Ph.D. thesis work involved the design of potent, dual PPAR α/γ and PPAR δ agonists. During postdoctoral training with Paul Torrence at the National Institutes of Health he worked on synthetic approaches to catalytic RNA/DNA chimeras directed against viral targets. In 1998 he accepted a position at 3-Dimensional Pharmaceuticals, which was merged into Johnson & Johnson Pharmaceutical Research and Development, L.L.C., in 2003. He now directs a medicinal chemistry team at the Spring House research site. He has delivered NCEs in the anti-inflammatory, analgesics, and cardiovascular therapeutic areas.

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