Potent Nonpeptide Antagonists of the Bradykinin B1 Receptor: Structure−Activity Relationship Studies with Novel Diaminochroman Carboxamides

Kaustav Biswas,*[,†] Aiwen Li,[†] Jian Jeffrey Chen,[†] Derin C. D'Amico,[†] Christopher Fotsch,[†] Nianhe Han,[†] Jason Human,[†] Qingyian Liu,[†] Mark H. Norman,[†] Bobby Riahi,[†] Chester Yuan,[†] Hideo Suzuki,[‡] David A. Mareska,[‡] James Zhan,[‡] David E. Clarke,[‡] Andras Toro,[‡] Robert D. Groneberg,[‡] Laurence E. Burgess,[‡] Dianna Lester-Zeiner,[†,§] Gloria Biddlecome,[†,§] Barton H. Manning,[∥] Leyla Arik,[∥] Hong Dong,[∥] Ming Huang,[∥] Augustus Kamassah,[∥] Richard Loeloff,[∥] Hong Sun,[∥] Feng-Yin Hsieh,[⊥] Gondi Kumar,[⊥] Gordon Y. Ng,[#] Randall W. Hungate,[†] Benny C. Askew,[†,§] and Eileen Johnson[∥]

Departments of Chemistry Research and Discovery, Pharmacokinetics and Drug Metabolism, Neuroscience, and Inflammation, Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320, and Array BioPharma, 3200 Walnut Street, Boulder, Colorado 80301

Received January 15, 2007

The bradykinin B1 receptor is induced following tissue injury and/or inflammation. Antagonists of this receptor have been studied as promising candidates for treatment of chronic pain. We have identified aryl sulfonamides containing a chiral chroman diamine moiety that are potent antagonists of the human B1 receptor. Our previously communicated lead, compound **2**, served as a proof-of-concept molecule, but suffered from poor pharmacokinetic properties. With guidance from metabolic profiling, we performed structure−activity relationship studies and have identified potent analogs of **2**. Variation of the sulfonamide moiety revealed a preference for 3- and 3,4-disubstituted aryl sulfonamides, while bulky secondary and tertiary amines were preferred at the benzylic amine position for potency at the B1 receptor. Modifying the β-amino acid core of the molecule lead to the discovery of highly potent compounds with improved in vitro pharmacokinetic properties. The most potent analog at the human receptor, compound **38**, was also active in a rabbit B1 receptor cellular assay. Furthermore, compound **38** displayed in vivo activity in two rabbit models, a pharmacodynamic model with a blood pressure readout and an efficacy model of inflammatory pain.

Introduction

Bradykinin (BK[a]), a positively charged nonapeptide, and kallidin (Lys-BK), along with their des-Arg$^{9/10}$ metabolites, are naturally occurring vasoactive peptide hormones called kinins. They have been implicated in vascular biology, inflammation, and pain, and are released at sites of tissue injury. These locally active peptides are produced by the proteolysis of inactive kininogen precursors by the enzymes plasma kallikrein and tissue kallikrein.[1,2]

The biological actions of the kinins are mediated by two G-protein coupled receptors, termed the B1 and B2 receptors.[3] BK and kallidin, which are short-lived peptides, are potent agonists of the B2 receptor, while the longer lasting des-Arg$^{9/10}$ metabolites activate the B1 receptor. Both B1 and B2 receptors are expressed on cells that initiate, exacerbate, and/or maintain inflammation and pain. Both receptors are also linked to phospholipase C activation, leading to intracellular Ca^{2+} generation by inositol 1,4,5-phosphate. The two receptors differ, however, in their regulation. B2 receptors are ubiquitously and constitutively expressed. B1 receptors, on the other hand, are absent or expressed at low levels in normal tissue, but are induced/upregulated following tissue injury and/or inflammation. After agonist stimulation, the B2 receptor is rapidly desensitized and internalized; which is in contrast to the B1 receptors that do not internalize but seem to translocate and aggregate.[4]

In vivo studies focusing on the role of B1 and B2 receptors in pain are consistent with their expression/regulation patterns. Preclinical pain models and knockout (KO) studies suggest that the B2 receptor plays a significant role in acute pain processing and the development of primary nociceptor sensitization and is the principal kinin receptor involved in cardiovascular and renal function.[1,5] In contrast, the B1 receptor plays a more limited role in acute pain processing but a prominent role in establishing and maintaining chronic pain.[6] Unlike B2 receptor agonists, B1 agonists are not algogenic in naïve animals. However, following tissue injury or inflammation, B1 agonists produce hyperalgesia. Furthermore, once the B1 receptor has been induced, antagonists are capable of reversing established pain. Differences between B1 and B2 receptors have also been observed in KO mice. B1 receptor deficient mice exhibit hypoalgesia to chemical and thermal noxious stimuli, show attenuated inflammatory responses and neutrophil accumulation, do not develop neuropathic pain associated with spinal nerve ligation or streptozotocin, and are normotensive.[7] This is in contrast to B2 KO mice that have salt-sensitive hypertension and, with the exception of lacking pain responses to BK, show normal pain responses.[8] Cardiovascular side effects of B2 antagonism could be a potential concern in the development of kinin receptor antagonists as therapeutic agents for pain and inflammation.

Evidence of the role of the B1 receptor in chronic pain has come from model studies with peptide antagonists. Synthetic

* To whom correspondence should be addressed. Tel.: 805-447-4836. Fax: 805-480-1337. E-mail: kbiswas@amgen.com.

[†] Department of Chemistry Research and Discovery, Amgen Inc.

[‡] Array BioPharma, Boulder, CO.

[§] Current address: Department of Hematology, Amgen Inc., Thousand Oaks, CA (G.B.); Department of Biology, Amgen Inc., 1120 Veterans Boulevard, South San Francisco, CA 94080 (D.L.-Z.); and Sereno Research Institute, Inc., One Technology Place, Rockland, MA 02370 (B.C.A.).

[∥] Department of Neuroscience, Amgen Inc., Thousand Oaks, CA.

[⊥] Department of Pharmacokinetics and Drug Metabolism, Amgen Inc.

[#] Department of Inflammation, Amgen Inc., Thousand Oaks, CA.

[a] Abbreviations: BK, bradykinin; KO, knockout; CL, clearance; RLMs, rat liver microsomes; SAR, structure−activity relationship; LPS, lipopolysaccharide; DAK, des-Arg-kallidin; DALK, des-Arg-Leu-kallidin; s.c., subcutaneous; DPPA, diphenyl phosphoryl azide; DBU, 1,8-diazabicyclo-[5.4.0]undec-7-ene; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; HOBt, *N*-hydroxybenzotriazole; dppp, diphenylphosphinopropane; HPMC, hydroxypropyl methylcellulose.

10.1021/jm070055c CCC: $37.00
Published on Web 04/05/2007

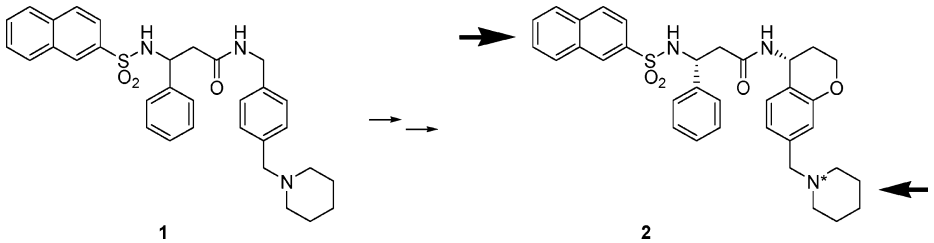


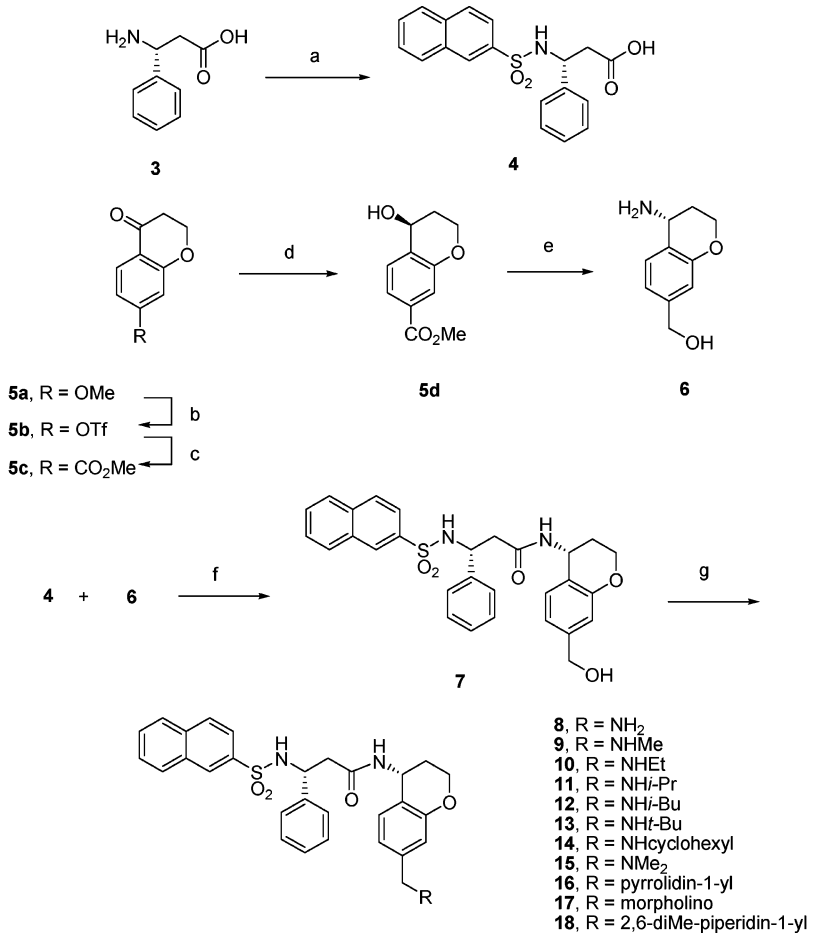
Figure 1. Evolution of lead aryl sulfonamide **2** through constraining the right-hand section of compound **1**. The bold arrows denote major sites of metabolism, including the highlighted benzylic amine (see reference 13).

peptide B1 receptor antagonists have been shown to reverse neurogenic pain induced by capsaicin as well as inflammatory pain induced by UV irradiation, carrageenan, complete Freund's adjuvant, or lipopolysaccharide (LPS).[9] The induction of functional B1 receptors and the reversal of established hyperalgesia by B1 antagonists also have been demonstrated in models of neuropathic pain including streptozotocin-induced diabetes, chronic constriction injury, and partial sciatic nerve lesion.[10] The preclinical data suggest that the B1 receptor is involved in the propagation, potentiation, and maintenance of chronic pain. The B1 receptor is, therefore, a novel and attractive therapeutic target for the treatment of chronic inflammatory and neuropathic pain conditions.

The above results, and successes with other peptide hormone-receptor systems such as angiotensin II, have also inspired efforts directed toward the development of small molecule B1 antagonists. Recently, many such reports have appeared in the literature.[11] The small molecule B1 antagonists in these studies have been shown to be active in pain and/or biochemical challenge assays, such as inflammatory hyperalgesia, neuropathic pain, spinal nociceptive reflex, or B1-mediated hypotension. These experiments support the notion that oral small molecule antagonists of the B1 receptor may have therapeutic potential for the treatment of chronic pain.

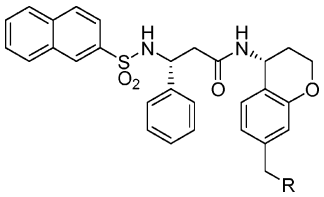
We have recently disclosed the results of our initial efforts in this arena with the identification of naphthyl sulfonamide **2**, which arose from constraining the right-hand portion of antagonist **1** (Figure 1).[12] Compound **2** is a potent small molecule antagonist of the B1 receptor (hB1 $K_i = 0.7$ nM). Cyclization of the right-hand amine moiety was shown to improve both binding affinity and cellular potency. These studies demonstrated a preference for (*R*)-stereochemistry at both the core amino acid side chain and at the chiral center on the bicyclic amine. With the potential cardiovascular implications of B2

Scheme 1. Preparation of Aryl Sulfonamides with Different Benzylic Amines in Table 1[a]



[a] Reagents and conditions: (a) 2-naphthalene sulfonyl chloride, $Na_2CO_3 \cdot H_2O$, 1,4-dioxane/water (7:3); (b) 48% HBr; Tf_2O, Et_3N, CH_2Cl_2; (c) 2 mol % $Pd(OAc)_2$, 2 mol % dppp, CO, DMF, MeOH, 65 °C; (d) 5 mol % (*R*)-methyl-CBS-oxazaborolidine, $BH_3 \cdot DMS$, toluene, −20 °C; (e) DPPA, DBU, toluene, 0 °C; $LiAlH_4$, THF; (f) EDC, HOBt, DMF; (g) MsCl, *i*-Pr_2NEt, DMF, 0 °C, then amine, 0 − 23 °C.

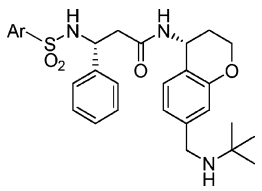
Table 1. SAR: Variation at the Benzylic Amine



cmpd	R	hB1 binding[a] $K_i \pm$ SEM (nM)	hB1 functional[a] $IC_{50} \pm$ SEM (nM)
2	piperidin-1-yl	0.7 ± 0.1	3.4 ± 0.1
7	OH	>50 000	>4000
8	NH_2	308 ± 9	352 ± 52
9	NHMe	138 ± 34	212 ± 21
10	NHEt	8.9 ± 1.3	60.2 ± 9.0
11	NH*i*-Pr	8.3 ± 1.3	45.9 ± 7.2
12	NH*i*-Bu	5.0 ± 0.6	10.5 ± 3.3
13	NH*t*-Bu	5.4 ± 0.7	17.0 ± 2.6
14	cyclohexylamino	21.8 ± 1.1	69.9 ± 17.1
15	*N*,*N*-diMe	84.8 ± 9.4	167 ± 50
16	pyrrolidin-1-yl	5.7 ± 0.9	14.8 ± 2.6
17	1-morpholino	3.7 ± 0.4	10.9 ± 1.2
18	2,6-diMe-piperidin-1-yl	13.4 ± 1.8	96.3 ± 15.6

[a] $n = 4$.

Table 2. SAR: Variation of Aryl Sulfonamides

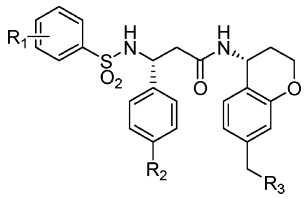


cmpd	Ar	hB1 binding[a] $K_i \pm$ SEM (nM)	hB1 functional[a] $IC_{50} \pm$ SEM (nM)
13	naphthyl	5.4 ± 0.7	17.0 ± 2.6
19	2-Cl−Ph	393 ± 31	2030 ± 191
20	3-Cl−Ph	27.7 ± 5.6	113 ± 12
21	4-Cl−Ph	132 ± 18	580 ± 117
22	2-CF_3-Ph	367 ± 59	2000 ± 326
23	3-CF_3-Ph	17.9 ± 2.7	78.4 ± 9.9
24	4-CF_3-Ph	77.6 ± 8.9	330 ± 48
25	4-*t*-Bu−Ph	24.8 ± 1.5	60.9 ± 12.5
26	3,4-di-Cl−Ph	7.6 ± 0.7	24.3 ± 5.5
27	2,4-di-Cl−Ph	127 ± 28	255 ± 8
28	3-Cl, 4-F−Ph	25.2 ± 3.5	203 ± 43
29	2-Me, 3-Cl−Ph	40.6 ± 6.4	207 ± 23
30	2,4-di-F, 5-Cl−Ph	20.6 ± 4.0	127 ± 25

[a] $n = 4$.

receptor antagonism in mind, we measured the selectivity of our antagonists for the human B1 over the human B2 receptor in binding assays. Compound **2** was highly selective at B1 over the B2 receptor (hB2 K_i > 50 μM). Although compound **2** served as a valuable tool as a proof-of-principle compound and offered a point of entry into this structural scaffold, it suffered from poor overall pharmacokinetic properties in rats (see Table 4). Incubation with rat and human microsomal preparations identified two major sites of metabolism (for human and rat liver microsomal data, see Table 4). Those included oxidation on the naphthalene moiety, as well as on the benzylic piperidine ring (Figure 1).[13] Therefore, we undertook a study aimed at identifying analogs of aryl sulfonamide **2**, where the sulfonamide and amine moieties were modified, which we hoped would result

Table 3. SAR: Variation of Core Amino Acid and Benzylic Amine



cmpd	R_1	R_2	R_3	hB1 binding[a] $K_i \pm$ SEM (nM)	hB1 functional[a] $IC_{50} \pm$ SEM (nM)
23	3-CF_3	H	NH*t*-Bu	17.9 ± 2.7	78.4 ± 9.9
31	3-CF_3	F	NH*t*-Bu	4.8 ± 0.7	9.9 ± 1.9
32	3-CF_3	CN	NH*t*-Bu	120 ± 25	566.2 ± 87.5
33	3-CF_3	F	$NH(CH_2)_2OMe$	14.0 ± 1.8	17.4 ± 1.2
34	3-CF_3	F	NH*i*-Pr	4.7 ± 0.3	20.1 ± 5.8
35	3-CF_3	F	NH*i*-Bu	5.9 ± 0.4	14.8 ± 4.0
36	3-CF_3	F	cyclobutylamino	10.0 ± 0.4	46.2 ± 8.1
37	3-CF_3	F	pyrrolidin-1-yl	5.5 ± 0.7	19.7 ± 2.0
38	3-CF_3	F	piperidin-1-yl	0.4 ± 0.1	0.8 ± 0.1
39	3,4-di-Cl	F	NH*t*-Bu	5.0 ± 0.7	9.6 ± 2.8
40	3,4-di-Cl	F	$NH(CH_2)_2OMe$	6.7 ± 0.9	9.4 ± 3.5

[a] $n = 4$.

in equipotent compounds with a better pharmacokinetic profile. This would enable us to begin the process of validation of this series of compounds as potential B1 antagonists with in vivo activity in inflammation models.

One area of concern in the development of in vivo models for testing B1 antagonists has been the relative lack of receptor homology across various species. Differences in the amino acid sequence of the B1 receptors from different species range between 69 and 97%. For example, the rat receptor is 71% homologous to the human counterpart,[14] while the rabbit B1 receptor has 82% homology to the human receptor.[15] As part of our pharmacological characterization of small molecule B1 antagonists, we also sought to establish robust preclinical models to evaluate the lead compounds identified through these studies. Therefore, we decided to measure the potency of small molecule antagonists of the human B1 receptor identified in this report in rodent and rabbit B1 receptor assays. This would allow us to identify compounds with cross-species activity that would enable preclinical determination of efficacy in animal models of pain and inflammation.

Herein, we report the synthesis and structure−activity relationship (SAR) studies with synthetic analogs of **2**, where we have maintained the chiral chroman diamine framework identified previously. We measured the affinity of the new compounds in a human B1 receptor binding assay and the functional potency in a B1 agonist-induced calcium flux assay in Chinese hamster ovary cells.[12] The results of this analysis led to the identification of potent compounds with better pharmacokinetic properties. Furthermore, one analog, compound **38**, was shown to be a potent antagonist of the rabbit B1 receptor, allowing us to test preclinical models in rabbits for evaluating in vivo efficacy of new compounds generated through this effort. Compound **38** was efficacious in a rabbit in vivo B1 agonist challenge pharmacodymanic assay as well as a rabbit inflammatory pain model.

Chemistry

The synthesis of these sulfonamides was based on a procedure of sequentially modifying the amino and the carboxyl termini of the core β-amino acid. Initially, we modified the benzylic

Table 4. Pharmacokinetic Parameters of Select Compounds

cmpd	$t_{1/2}$[a] (h)	CL[a] (mL/min/kg)	V_{ss}[a] (mL/kg)	F[b] (%)	HLM[e] (μL/min/mg)	RLM[e] (μL/min/mg)
2[c]	1.4	84.1	7427	4[d]	688	390
33	3.4 ± 1.3	39.4 ± 6.2	8085 ± 3154	49	310	265
37	3.8 ± 0.5	51.3 ± 4.2	13 669 ± 485	41	355	158
38	2.5 ± 0.4	24.8 ± 2.6	4636 ± 863	13	330	216

[a] Dosed i.v. at 1 mg/kg, formulated in 100% DMSO, $n = 3$, reported with SEM. [b] Dosed orally at 10 mg/kg, formulated in 1% Tween 80, 2% HPMC, $n = 3$, interanimal variability <20%. [c] For **2**, $n = 2$, interanimal variability <20%. [d] Dosed orally at 3 mg/kg, formulated in 1% Tween 80, 2% HPMC.[e] $n = 1$.

amine moiety on **2** (Figure 1). The synthetic route is described in Scheme 1. Commercially available (*R*)-3-amino-3-phenylpropionic acid (**3**) was treated with 2-naphthalene sulfonyl chloride under Schotten−Baumann conditions to afford acid **4** in 46% yield. The synthesis of the bicyclic amino alcohol **6** has been described previously,[12] using the Corey−Bakshi−Shibata oxazaborolidine reduction[16] of chromanone **5c**, which was obtained from Pd(II)-mediated carbonylation of triflate **5b**. Subsequent azide displacement with diphenylphosphoryl azide to set the (*R*)-stereochemistry at the benzylic chiral center and lithium aluminum hydride reduction afforded amino alcohol **6**. Carbodiimide-mediated coupling between acid **4** and amino alcohol **6** under the agency of hydroxybenzotriazole afforded **7** in 61% yield. Benzylic alcohol **7** was used as the common intermediate in the one-pot two-step synthesis of the analogous benzylic amines. The alcohol was converted to the mesylate with methanesulfonyl chloride and diisopropylethylamine, and the crude product was treated in situ with a primary or secondary amine to afford the corresponding benzylic amines **8**−**18**, in yields ranging from 37 to 72%.

The compounds in Table 2 were prepared in a similar fashion, using different commercially available aryl sulfonyl chlorides instead of 2-naphthalene sulfonyl chloride in the first sulfonamidation step with amino acid **3**. Similarly, the synthesis of compounds in Table 3 were initiated from commercially available β-amino acids, (*R*)-3-amino-3-(4-fluorophenyl)-propionic acid and (*R*)-3-amino-3-(4-cyanophenyl)-propionic acid (for **32**), using the same synthetic sequence.

Results and Discussion

The compounds synthesized as part of this study were tested in a human B1 receptor binding assay and a human B1 agonist-induced cellular calcium flux assay, which have been described previously.[12] These data are reported as $K_i \pm$ SEM and $IC_{50} \pm$ SEM, respectively, in Tables 1−3. The K_i and IC_{50} values of lead sulfonamide **2** are included in Table 1 for comparison. To simplify the discussion, the SAR studies disclosed below were based on the IC_{50} data from the cellular calcium flux assay, but similar trends were observed for the K_i values for the receptor binding assay. As seen with this series previously, human B2 receptor binding assays determined that all compounds were selective for the B1 receptor (hB2 K_is > 20 μM, data not shown).

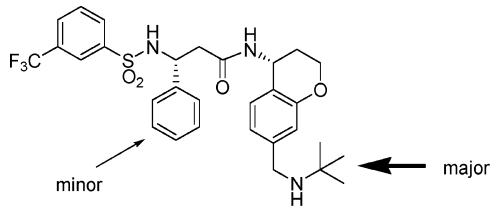
We began our studies by examining the effect of modifying the benzylic amine moiety of compound **2** in an effort to identify suitable replacements that retained functional potency (Table 1). The alcohol **7** had no measurable activity in our B1 binding and functional assays, demonstrating the requirement of a basic amine at that site for activity at the receptor. The primary amine **8** showed moderate activity ($IC_{50} = 352$ nM). We next examined the effect of alkyl substituents at the benzylic amine site (compounds **9**−**14**). With these secondary amines, we observed a trend toward increased potency with increasing bulk and lipophilicity. Addition of a methyl substituent on the amine did not significantly improve potency (**9**, $IC_{50} = 212$ nM), but the ethyl (**10**) and isopropyl (**11**) amino analogues had improved IC_{50}s of 60.2 and 45.9 nM, respectively. Increasing the bulk at the basic nitrogen led to compounds with higher potency. The more lipophilic *iso*-butylamino (**12**) and *tert*-butylamino (**13**) compounds had IC_{50}s of 10.5 and 17 nM, respectively, but were still less potent than **2**. Introduction of a different mode of branching with cyclohexyl amine, as in **14**, however, had the effect of a modest decrease in potency ($IC_{50} = 69.9$ nM).

We also incorporated secondary amines into the synthetic procedure, affording the corresponding tertiary benzylic amine analogs of **2** (compounds **15**−**18**). The functional assay data from these compounds followed the similar trend seen above with a preference for greater lipophilicity. The *N*,*N*-dimethyl amino compound **15** showed a loss in potency compared to compound **2**, with an IC_{50} of 167 nM. However, similar to the *iso*-butylamino (**12**) and *tert*-butylamino (**13**) compounds described above, addition of lipophilicity increased potency. The cyclic pyrrolidinyl and morpholino compounds **16** and **17** were more potent than **15**, with IC_{50}s of 14.8 and 10.9 nM, respectively. The 2,6-dimethylpiperidinyl analog, compound **18** ($IC_{50} = 96.3$ nM) showed almost a 30-fold loss in potency compared to the parent compound **2**.

The SAR studies with different amines in Table 1 revealed a preference for bulkier amines at the benzylic position of these compounds. We next examined the effect of modifications of the aryl sulfonamide on the functional potency at the human B1 receptor. Among the potent amine analogs identified in Table 1, we maintained the *tert*-butylamino functionality constant, while varying the substitution pattern on the arylsulfonamide fragment.[17] These data, which we hoped would reveal the preferred patterns of lipophilic substitution in lieu of the naphthyl moiety, are illustrated in Table 2. For reference, compound **13** is also included in Table 2.

In general, replacement of the naphthalene fragment with a benzene ring substituted with lipophilic functional groups resulted in a loss of potency. Despite this, certain trends emerged from this SAR study. Monosubstitution of the phenyl ring with chloro or trifluoromethyl groups is clearly preferred in the 3-position (compounds **20** and **23**, IC_{50}s of 113 and 78.4 nM, respectively). Substituents in the 2-position of the phenyl ring resulted in the most significant decreases in potency, with IC_{50}s > 1 μM (compounds **19** and **22**). Substitution at the 4-position resulted in compounds with a range of activities, with the pattern of 4-*t*-Bu > 4-CF_3 > 4-Cl substituents being preferred in terms of potency (compounds **25**, **24**, and **21**, respectively). The 4-*tert*-butylphenyl compound **25** exhibited an $IC_{50} = 60.9$ nM.

We subsequently examined the effect of poly-substitution on the arylsulfonamide moiety. The disubstituted phenylsulfonamides **27**−**29** demonstrated a loss in potency over naphthylsulfonamido compound **13**, with IC_{50}s in the 200 nM range. One exception was the 3,4-dichlorophenylsulfonamide compound **26** ($IC_{50} = 24.3$ nM), which was comparable to compound **13**, exhibiting similar cellular activity. Trisubstituted



23

Figure 2. Sites of metabolism of compound **23** in RLMs, denoted by arrows.

analog **30** showed an approximately 6-fold loss in potency compared to that of naphthyl compound **13**.

While our efforts at modifying the naphthalene and piperidinyl fragments of **2** resulted in the identification of preferred substitution patterns, these compounds displayed diminished functional potency at the B1 receptor. To proceed further in our studies, we decided to study the in vitro microsomal stability of a representative new analog. We profiled the 3-trifluoromethylphenyl compound **23** to examine possible sites of metabolism to guide further modifications (Figure 2). The major metabolites in rat liver microsomes (RLMs) were identified to be oxidation products at the benzylic *tert*-butyl group. It is worth noting that while replacing the piperidinyl functionality in **2** with the *tert*-butyl in the compounds in Table 2 did not abrogate metabolism at the benzylic center, this series of compounds did help us in identifying the preferred pattern of substitution on the aryl sulfonamide fragment of this antagonist series. Interestingly, a minor metabolite was identified that possessed a hydroxyl group at the central β-amino acid core. Because this is a portion of the molecule that we had not yet modified in our SAR program, we undertook a brief examination of changes in the core amino acid. These compounds are described in Table 3. Compound **23** is also included in Table 3 for reference.

Two compounds with the core phenyl ring substituted with electron withdrawing groups at the 4-position were initially prepared. Compound **31**, where a (*R*)-3-amino-3-(4-fluorophenyl)-propionic acid unit was incorporated instead of the phenyl moiety, as in compound **23**, increased potency by 8-fold (IC_{50} = 9.9 nM). However, compound **32**, with a 4-cyanophenylpropionic acid unit, exhibited diminished potency, with a 7-fold loss compared to **23**. Because we gained potency with the incorporation of a fluorine atom in the 4-position of the core amino acid phenyl moiety, we proceeded to synthesize more analogs with this fragment included in the molecule.

Recognizing that the *tert*-butylamino group was metabolized in compound **23**, we modified the benzylic amine moiety with a series of different amine functionalities (compounds **33**−**38**). The linear secondary amine **33** showed a functional IC_{50} of 17.4 nM. Branched amines also were potent, with the isopropyl amine **34** (IC_{50} = 20.1 nM) showing a similar 4-fold gain in potency over **23**, as did the isobutyl amine **35**, with an IC_{50} = 14.8 nM. The cyclobutylamino compound **36** was slightly less potent, being less than 2-fold better than compound **23**. Cyclic tertiary benzylic amines were also synthesized. The pyrrolidinyl analog **37** was 4-fold more potent than **23**. The homologous six-membered ring containing piperidinyl analog **38** was very potent, with an IC_{50} of 0.8 nM, a gain of an order of magnitude over **23** and a potency similar to our original lead, compound **2**.

Because the 3,4-dichlorophenylsulfonamide unit was also identified in Table 2 as a potent naphthalene replacement, we probed the combination of compounds with 3,4-dichlorophenylsulfonamide with the core 4-fluorophenyl amino acid (compounds **39** and **40**). Compound **39** was 2.5-fold more potent than corresponding phenyl amino acid compound **26**, with an IC_{50} = 9.6 nM. Inclusion of a linear amine at the benzylic position in compound **40** led to a compound of similar activity, with an IC_{50} = 9.4 nM.

Table 5. Cross-Species Antagonist Activity of Select New Compounds

	hB1			
cmpd	$K_i \pm$ SEM[a] (nM)	$IC_{50} \pm$ SEM[a] (nM)	Rat B1 $K_i \pm$ SEM (nM)	Rabbit B1 $IC_{50} \pm$ SEM (nM)
33	14.0 ± 1.8	17.4 ± 1.2	208 ± 18^a	1685 ± 74^b
37	5.5 ± 0.7	19.7 ± 2.0	151 ± 17^b	450.5 ± 20.6^b
38	0.4 ± 0.1	0.8 ± 0.1	127 ± 28^c	10.4 ± 7.1^d

[a] $n = 4$. [b] $n = 3$. [c] $n = 6$. [d] $n = 15$.

In summary, the SAR studies reported above led to the identification of compounds with similar potency to compound **2**. We were guided in our design by metabolism data, which identified major sites of oxidation. We identified a preference for bulky secondary or tertiary amines at the benzylic position and a preference for phenyl sulfonamides substituted at the 3- and 3,4- positions with lipophilic groups. Notably, modification of the core amino acid at the 4-position of the phenyl ring with a fluorine atom led to increases in functional potency, with compound **38** being the most active compound identified so far in our functional assay.

Pharmacokinetics

We note parenthetically that compound **38** also possesses the same piperidinyl moiety as the basic amine as our original lead, compound **2**. Because these studies were designed to find potent compounds with better pharmacokinetic profiles, we evaluated compound **38** in i.v. and oral pharmacokinetic studies in Sprague−Dawley rats, as well as in vitro microsomal preparations. We also included compounds **2**, **33**, and **37** in this study, summarized in Table 4.

The compounds described in this exploration had longer half-lives ($t_{1/2}$) compared to compound **2**. Whereas **2** had a $t_{1/2}$ of 1.4 h, compounds **38** and **37** had a $t_{1/2}$ of 2.5 and 3.8 h, respectively. Compound **38** exhibited a significantly lowered rate of clearance (CL) of 24.8 mL/min/kg (3.5-fold lower than compound **2**). Compounds **33** and **37** were well absorbed, with oral bioavailabilities (*F*) of 49 and 41%, respectively. Compound **38** demonstrated an *F* of 13%. We also examined the stability of these new analogs in liver microsomes for an in vitro estimate of differences in oxidative metabolism compared to compound **2**. The data with human liver microsomes and RLMs for the compounds in Table 4 show an approximately 2-fold decrease in CL in both species from the corresponding data for compound **2**. Therefore, in summary, the SAR studies described above have led to the identification of compounds with better pharmacokinetic profiles as compared to the initial lead, compound **2**.

Pharmacology. As mentioned previously, one goal of this study was to establish robust preclinical models for evaluating new analogs in an in vivo setting. Noting the difference in homology of the B1 receptor in different species, we measured the activity of some of the more potent analogs (compounds **33**, **37**, and **38**) in rodent and rabbit B1 receptor assays (Table 5). The compounds had moderate affinity for the rat B1 receptor (K_is > 100 nM), complicating evaluation in existing rat models of inflammation. While compounds **33** and **37** had low to moderate activity in the rabbit B1 cellular assay, compound **38** had good functional potency, with an IC_{50} of 10.4 nM in the rabbit assay. Therefore, compound **38** was selected as a proof-

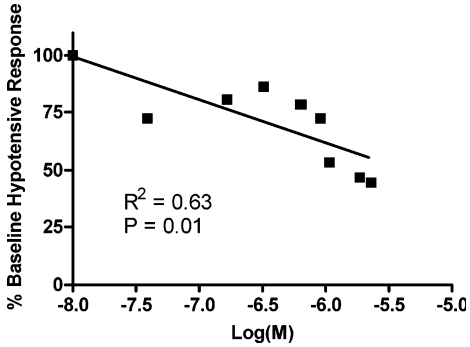


Figure 3. Compound **38** reduces DAK-induced hypotension. Each data point reflects the % hypotensive response due to DAK administration either at 30 min or at 75 min post-administration of antagonist **38** (the *x*-axis represents the plasma concentration at those time points).

of-principle compound for the evaluation of in vivo activity in two rabbit models, a pharmacodynamic model with a blood pressure readout and an efficacy model of inflammatory pain.

Following administration of systemic bacterial LPS, the B1 receptor is up-regulated and a B1 agonist (e.g., des-Arg^{10}-kallidin; DAK) induces a hypotensive response.[18,19] As our first model, we employed a hypotension-reversal paradigm in rabbits to measure the in vivo activity of our small molecule B1 antagonists, as has been reported previously for another series of small molecule B1 antagonists.[11b] Rabbits were treated with five different doses of compound **38** (1, 3, 5, 8, and 10 mg/kg, subcutaneous (s.c.), see Experimental Section), followed by repeat administration of the agonist DAK at two different time points. Blood samples were collected prior to the DAK administrations to obtain plasma concentrations of compound **38**, which were correlated with the hypotensive responses (Figure 3). Compound **38** significantly reduced the B1 agonist-induced hypotension in a plasma concentration-dependent manner and showed a maximum decrease of 47% in the hypotensive response at the highest plasma concentrations. Compound **38** is highly protein-bound (rabbit plasma protein binding = 94.5%), which might explain the difference in efficacy when compared to its in vitro potency at the rabbit receptor.

We next examined compound **38** in a second in vivo model. In an efficacy model of inflammatory pain, mechanical hyperalgesia was induced in the rabbit hind paw using local injection of carrageenan.[20] Systemic (s.c.) injection of compound **38** dose-dependently reversed hyperalgesia when administered 2 h after carrageenan (Figure 4). A dose of 10 mg/kg resulted in 45.5% reversal of hyperalgesia, as compared with vehicle. The plasma levels of the compound also show a corresponding increase. By comparison, the reference peptide B1 antagonist des-Arg^{10}-Leu^{9}-kallidin (DALK; 10 mg/kg, s.c.) reversed hyperalgesia by 78% when administered 2.5 h after carrageenan.

Conclusion

In summary, we have described an SAR study guided by metabolic profiling of lead compound **2**, a potent antagonist of the human BK B1 receptor. Replacement of piperidinyl benzylic amine and naphthalene sulfonamide moieties revealed a preference for bulky amines and 3-/3,4-disubstituted phenyl sulfonamides for B1 antagonism. However, replacement of the naphthalene sulfonamide fragment led to an overall loss in cellular potency. Based on metabolite identification studies of compound **23**, a fluorine atom was introduced in the phenyl ring in the core β-amino acid, and a series of benzylic amine substituents with this new core were synthesized. A number of potent analogs of **2** were discovered with this modification. Compound **38**, containing a 3-trifluoromethylphenyl sulfonamide and a piperidinyl amine, was identified as the most potent analog in our human B1 functional cellular assay in this series of compounds (hB1 IC_{50} = 0.8 nM). As a result of these studies, new analogs with better in vitro and in vivo pharmacokinetic profiles than compound **2** were also discovered. These compounds had longer half-lives than compound **2**, oral bioavailabilities ranging from 13 to 49% in rats, and decreased oxidative metabolism, as measured by increased stability in liver microsomal preparations. To identify a preclinical species for determining in vivo efficacy of this series of analogs, we measured the antagonist activity of a few potent compounds (in the hB1 assay) against rat and rabbit B1 receptors. Compound **38** was a potent antagonist of the rabbit B1 receptor (rabbit B1 IC_{50} = 10.4 nM) and was selected as a test compound to determine preclinical efficacy in animal models. Evaluation of compound **38** in two rabbit in vivo models revealed that it is efficacious in preventing B1 agonist-induced hypotension and in reversing inflammatory pain in a dose-dependent manner. Further studies in this program are in progress and will be reported elsewhere in due course.

Experimental Section

Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. (*R*)-3-amino-3-phenylpropionic acid (**3**), (*R*)-3-amino-3-(4-fluorophenyl)-propionic acid, and (*R*)-3-amino-3-(4-cyanophenyl)-propionic acid were purchased from Peptech Corporation, Burlington, MA. Anhydrous solvents were obtained from Aldrich or EM Science and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen atmosphere. All final compounds were purified to >95% purity, as determined by high-performance liquid chromatography (HPLC). Silica gel chromatography was performed using either glass columns packed with silica gel (230−400 mesh, EMD Chemicals, Gibbstown, NJ) or prepacked silica gel cartridges (Biotage). Thin-layer chromatography (TLC) was performed using EMD 250 μm prescored silica gel 60 F_{254} plates. NMR spectra were recorded on a Bruker DRX 400 MHz or a Bruker DRX 300 MHz spectrometer. Low-resolution mass spectral data (MS) were determined on a Perkin-Elmer SCIEX API 165 mass spectrometer using ES ionization modes (positive). High-resolution mass spectral data were acquired on a 7 T Bruker Apex IV Fourier-Transform Mass Spectrometer (FTMS) using ES ionization modes. Combustion analysis was performed by Atlantic Microlab, Norcross, GA, and is reported as a mean of duplicate values.

(*R*)-3-(Naphthalene-3-sulfonamido)-3-phenylpropanoic Acid (4). A solution of (*R*)-3-amino-3-phenylpropanoic acid **3** (5.12 g, 31 mmol) and 2-naphthalenesulfonyl chloride (7.0 g, 31 mmol) in 1,4-dioxane (70 ml) and water (30 ml) was treated with sodium carbonate monohydrate (9.6 g, 77 mmol). The reaction was stirred at 23 °C. After 15 h, the solution was diluted with 10% hydrochloric acid solution (250 ml) and extracted with EtOAc (2 × 250 ml). The combined organic layers were washed with brine (250 ml), dried over $MgSO_4$, concentrated in vacuo, and purified by silica gel chromatography (eluant: 5% methanol/dichloromethane), affording the product (5.1 g, 46%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.25 (1H, br s), 8.45 (1H, d, J = 8.2 Hz), 8.17 (1H, s), 7.99 (1H, d, J = 7.8 Hz), 7.87−7.96 (2H, m), 7.57−7.67 (3H, m), 7.13 (2H, d, J = 7.2 Hz), 7.01 (2H, t, J = 7.5 Hz), 6.93 (1H, d, J = 7.2 Hz), 4.71 (1H, dd, J = 14.2, 7.1 Hz), 2.64 (1H, dd, J = 15.5, 7.4 Hz), 2.58 (1H, dd, J = 15.5, 7.6 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 170.9, 140.4, 138.2, 133.7, 131.3, 128.9, 128.7, 128.3, 127.7, 127.5, 127.1, 127.0, 126.8, 126.5, 122.0, 54.6, 42.0. MS (ESI, pos. ion) *m/z* 356.1 (M + H).

(*R*)-*N*-((*R*)-7-(Hydroxymethyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (7). A solution of (*R*)-3-(naphthalene-3-sulfonamido)-3-phenylpropanoic acid **4** (3.81 g, 10.7

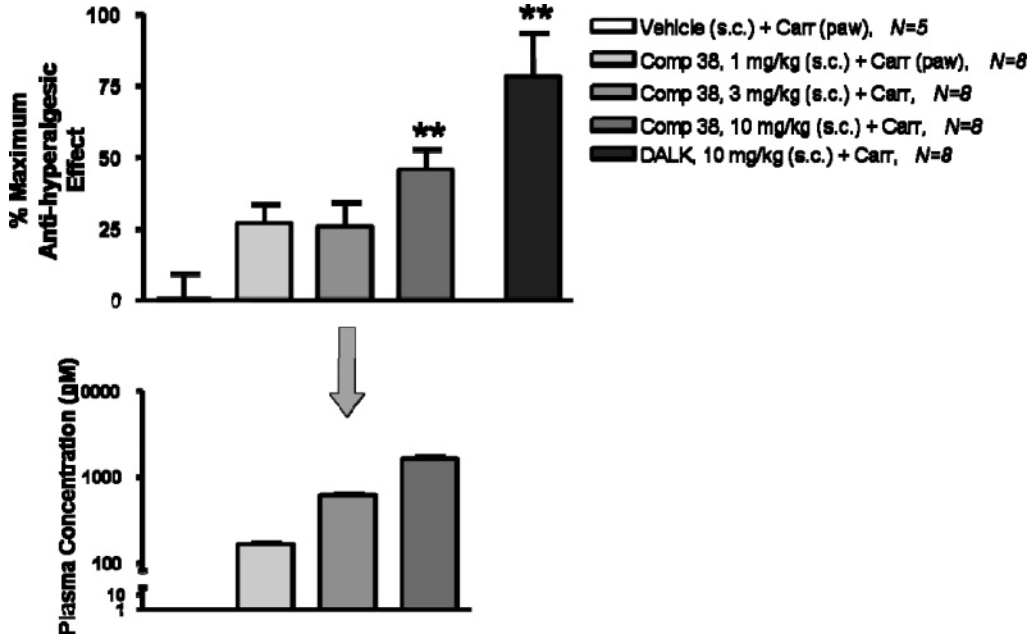


Figure 4. Antinociceptive effects of test compounds in a rabbit inflammatory pain model (carrageenan-induced mechanical hyperalgesia). Plasma levels of compound **38** are also included for each dose.

mmol) and (*R*)-(4-aminochroman-7-yl)methanol **6** (2.40 g, 13.4 mmol) in DMF (30 ml) was treated with 1-hydroxybenzotriazole (2.17 g, 16.1 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (3.08 g, 16.1 mmol). The reaction was stirred at 22 °C. After 15 h, the solution was diluted with EtOAc (250 ml) and washed with 10% hydrochloric acid solution (100 ml) and brine (100 ml), dried over $MgSO_4$, concentrated in vacuo, and purified by silica gel chromatography (eluant: 2% methanol/dichloromethane), affording the product (3.39 g, 61%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.42 (1H, d, J = 8.8 Hz), 8.22 (1H, s), 8.17 (1H, d, J = 8.4 Hz), 8.02 (1H, d, J = 7.8 Hz), 7.95 (2H, t, J = 6.8 Hz), 7.60−7.68 (3H, m), 7.11−7.20 (2H, m), 7.01−7.09 (3H, m), 6.60 (1H, s), 6.50 (1H, d, J = 8.0 Hz), 6.12 (1H, d, J = 8.0 Hz), 5.05 (1H, t, J = 5.8 Hz), 4.69−4.81 (2H, m), 4.32 (2H, d, J = 5.7 Hz), 4.03 (2H, t, J = 5.2 Hz), 2.57 (1H, s), 2.45 (1H, s), 1.79−1.87 (1H, m), 1.60−1.69 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.7, 154.2, 142.9, 140.3, 138.2, 133.7, 131.4, 128.9, 128.7, 128.3, 127.6, 127.5, 127.2, 127.0, 126.8, 122.0, 121.1, 118.0, 113.7, 63.2, 62.2, 55.0, 43.3, 41.8, 28.6. MS (ESI, pos. ion) *m/z* 517.1 (M + H). Anal. Calcd for $C_{29}H_{28}N_2O_5S$: C, 67.72; H, 5.46; N, 5.42. Found: C, 67.14; H, 5.38; N, 5.48.

General Procedure A for Conversion of Alcohol 7 to Amines 8−18. A solution of (*R*)-*N*-((*R*)-7-(hydroxymethyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide **7** (289 mg, 559 μmol) and *N*,*N*-diisopropylethylamine (244 μL, 181 mg, 1399 μmol) in DMF (6 ml) was cooled to 0 °C and treated with methanesulfonyl chloride (52 μl, 77 mg, 671 μmol). The reaction was stirred at 0 °C. After 45 min, the corresponding amine (2238 μmol) was added, and the reaction was warmed to 23 °C. After a further 90−180 min, the solution was diluted with EtOAc (100 ml) and washed with saturated sodium bicarbonate solution (50 ml), water (50 ml) and brine (50 ml), dried over $MgSO_4$, concentrated in vacuo, and purified by silica gel chromatography (eluant: 4% methanol/dichloromethane), which afforded the product as a white solid in 37−72% yields.

(*R*)-*N*-((*R*)-7-(Aminomethyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (8). The title compound was prepared according to general procedure A, employing 30% ammonium hydroxide as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.22 (1H, s), 8.18 (1H, d, J = 8.2 Hz), 8.02 (1H, d, J = 7.8 Hz), 7.95 (3H, t, J = 7.7 Hz), 7.59−7.68 (3H, m), 7.15 (2H, d, J = 5.9 Hz), 7.00−7.10 (3H, m), 6.65 (1H, s), 6.53 (2H, d, J = 8.0 Hz), 6.11 (1H, d, J = 7.8 Hz), 4.78 (1H, dd, J = 9.2, 6.1 Hz), 4.67−4.75 (1H, m), 4.03 (2H, t, J = 5.0 Hz), 3.57 (1H, s), 2.52−2.61 (1H, m), 2.39−2.49 (1H, m), 1.77−1.89 (1H, m), 1.59−1.69 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.8, 154.3, 143.3, 140.4, 138.3, 133.8, 131.4, 129.0, 128.8, 128.5, 128.4, 127.7, 127.6, 127.2, 127.1, 126.9, 122.1, 121.1, 119.0, 114.6, 63.3, 55.1, 44.7, 43.4, 41.9, 28.7. HRMS (ESI, pos. ion) calcd for $C_{29}H_{29}N_3O_4S$, 516.1944 (M + H); found, 516.1949. Anal. Calcd for $C_{29}H_{29}N_3O_4S\cdot 1.2H_2O$: C, 64.83; H, 5.89; N, 7.82. Found: C, 64.76; H, 5.38; N, 7.32.

(*R*)-*N*-((*R*)-7-((Methylamino)methyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (9). The title compound was prepared according to general procedure A, employing methylamine, 2.0 M in THF, as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.22 (1H, s), 8.18 (1H, d, J = 8.2 Hz), 8.02 (1H, d, J = 7.8 Hz), 7.95 (2H, t, J = 7.6 Hz), 7.60−7.68 (3H, m), 7.15 (2H, d, J = 6.1 Hz), 7.01−7.09 (3H, m), 6.62 (1H, s), 6.51 (1H, d, J = 7.2 Hz), 6.13 (1H, d, J = 7.8 Hz), 4.59−4.86 (2H, m), 4.03 (2H, t, J = 5.1 Hz), 3.49 (2H, s), 2.52−2.63 (1H, m), 2.37−2.49 (1H, m), 2.20 (3H, s), 1.77−1.87 (1H, m), 1.60−1.69 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.8, 154.3, 140.7, 140.4, 138.3, 133.8, 131.4, 129.0, 128.8, 128.5, 128.4, 127.7, 127.6, 127.2, 127.1, 126.9, 122.1, 121.2, 119.8, 115.3, 63.3, 55.1, 54.3, 43.4, 41.9, 35.1, 28.7. HRMS (ESI, pos. ion) calcd for $C_{30}H_{31}N_3O_4S$, 530.2100 (M + H); found, 530.2104. Anal. Calcd for $C_{30}H_{31}N_3O_4S\cdot 0.5H_2O$: C, 66.89; H, 5.99; N, 7.80. Found: C, 66.71; H, 5.82; N, 7.60.

(*R*)-*N*-((*R*)-7-((Ethylamino)methyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (10). The title compound was prepared according to general procedure A employing ethylamine, 2.0 M in THF, as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.21 (2H, s), 8.02 (1H, d, J = 7.4 Hz), 7.95 (2H, t, J = 8.3 Hz), 7.59−7.69 (3H, m), 7.16 (2H, d, J = 5.9 Hz), 7.00−7.09 (3H, m), 6.73 (1H, s), 6.60 (1H, d, J = 7.6 Hz), 6.17 (1H, d, J = 8.0 Hz), 4.64−4.87 (2H, m), 4.00−4.11 (2H, m), 3.72 (2H, s), 2.52−2.69 (3H, m), 2.41−2.48 (1H, m), 1.79−1.88 (1H, m), 1.61−1.71 (1H, m), 1.07 (3H, t, J = 7.0 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.9, 154.3, 140.4, 138.3, 133.8, 131.4, 129.0, 128.8, 128.6, 128.4, 127.7, 127.6, 127.2, 127.1, 126.9, 122.4, 122.1, 120.4, 116.2, 63.5, 55.1, 50.6, 43.4, 42.0, 41.9, 28.5, 13.01. HRMS (ESI, pos. ion) calcd for $C_{31}H_{33}N_3O_4S$, 544.2256 (M + H); found, 544.2264.

(*R*)-*N*-((*R*)-7-((Isopropylamino)methyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (11). The title compound was prepared according to general procedure A, employing isopropylamine as the amine. ^{1}H NMR (400 MHz, DMSO-d_6)

δ ppm 8.46 (1H, br s), 8.17−8.24 (2H, m), 8.02 (1H, d, J = 7.6 Hz), 7.95 (2H, t, J = 8.0 Hz), 7.60−7.68 (3H, m), 7.11−7.20 (2H, m), 7.01−7.08 (3H, m), 6.72 (1H, s), 6.59 (1H, d, J = 7.6 Hz), 6.16 (1H, d, J = 7.8 Hz), 4.70−4.81 (2H, m), 4.00−4.09 (2H, m), 3.66 (2H, s), 2.72−2.82 (1H, m), 2.53−2.62 (1H, m), 2.42−2.49 (1H, m), 1.79−1.88 (1H, m), 1.61−1.71 (1H, m), 1.03 (6H, d, J = 5.3 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.8, 154.2, 140.4, 138.3, 133.7, 131.4, 128.9, 128.7, 128.5, 128.3, 127.6, 127.5, 127.1, 127.0, 126.8, 122.0, 120.1, 115.8, 63.4, 55.1, 47.3, 43.3, 41.8, 28.5, 21.2. HRMS (ESI, pos. ion) calcd for $C_{32}H_{35}N_3O_4S$, 558.2412 (M + H); found, 558.2421. Anal. Calcd for $C_{32}H_{35}N_3O_4S\cdot 0.9H_2O$: C, 66.97; H, 6.46; N, 7.32. Found: C, 66.62; H, 6.17; N, 7.23.

(*R*)-*N*-((*R*)-7-((Isobutylamino)methyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (12). The title compound was prepared according to general procedure A, employing isobutylamine as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.43 (1H, br s), 8.22 (1H, s), 8.19 (1H, d, J = 8.2 Hz), 8.03 (1H, d, J = 7.8 Hz), 7.96 (2H, t, J = 7.8 Hz), 7.60−7.69 (3H, m), 7.16 (2H, d, J = 6.5 Hz), 7.00−7.09 (3H, m), 6.66 (1H, s), 6.55 (1H, d, J = 7.8 Hz), 6.14 (1H, d, J = 7.8 Hz), 4.69−4.82 (2H, m), 4.00−4.12 (2H, m), 3.59 (2H, s), 3.18 (1H, s), 2.52−2.63 (1H, m), 2.40−2.49 (1H, m), 2.26 (2H, d, J = 6.7 Hz), 1.79−1.88 (1H, m), 1.61−1.72 (1H, m), 0.85 (6H, d, J = 6.7 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.8, 154.3, 140.4, 138.3, 133.8, 131.4, 129.0, 128.8, 128.5, 128.4, 127.7, 127.6, 127.2, 127.1, 126.9, 122.1, 121.5, 119.8, 115.4, 63.4, 56.0, 55.1, 52.1, 48.5, 43.4, 41.9, 28.6, 27.5, 20.6. HRMS (ESI, pos. ion) calcd for $C_{33}H_{37}N_3O_4S$, 572.2568 (M + H); found, 572.2577. Anal. Calcd for $C_{33}H_{37}N_3O_4S\cdot 0.5H_2O$: C, 68.25; H, 6.60; N, 7.24. Found: C, 67.93; H, 6.31; N, 7.15.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (13). The title compound was prepared according to general procedure A, employing *tert*-butylamine as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.48 (1H, d, J = 8.2 Hz), 8.18−8.26 (2H, m), 8.01 (1H, d, J = 7.8 Hz), 7.95 (2H, t, J = 8.5 Hz), 7.59−7.68 (3H, m), 7.12−7.20 (2H, m), 7.00−7.10 (3H, m), 6.82 (1H, s), 6.63−6.72 (1H, m), 6.21 (1H, d, J = 7.8 Hz), 4.69−4.83 (2H, m), 3.99−4.13 (2H, m), 3.77 (2H, s), 2.53−2.64 (1H, m), 2.47 (1H, d, J = 6.5 Hz), 1.84 (1H, s, J = 11.2 Hz), 1.61−1.73 (1H, m), 1.22 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 168.0, 154.3, 140.4, 138.3, 133.8, 131.4, 129.0, 128.8, 128.6, 128.4, 127.7, 127.6, 127.2, 127.1, 126.9, 126.9, 122.1, 63.6, 55.2, 54.9, 43.4, 42.0, 28.5. HRMS (ESI, pos. ion) calcd for $C_{33}H_{37}N_3O_4S$, 572.2568 (M + H); found, 572.2574.

(*R*)-*N*-((*R*)-7-((Cyclohexylamino)methyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (14). The title compound was prepared according to general procedure A, employing cyclohexylamine as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.47 (1H, br s), 8.22 (2H, t, J = 3.9 Hz), 8.02 (1H, d, J = 7.6 Hz), 7.95 (2H, t, J = 8.2 Hz), 7.60−7.69 (3H, m), 7.16 (2H, d, J = 6.3 Hz), 7.00−7.10 (3H, m), 6.74 (1H, s), 6.61 (1H, d, J = 7.6 Hz), 6.17 (1H, d, J = 7.8 Hz), 4.71−4.82 (2H, m), 4.01−4.11 (2H, m), 3.73 (2H, s), 2.53−2.63 (1H, m), 2.43−2.49 (1H, m), 1.78−1.95 (3H, m), 1.63−1.73 (3H, m), 1.50−1.58 (1H, m), 1.06−1.21 (6H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.9, 154.3, 140.4, 138.3, 133.8, 131.4, 129.0, 128.8, 128.6, 128.4, 127.7, 127.6, 127.2, 127.1, 126.9, 122.1, 120.3, 116.0, 63.5, 55.1, 55.0, 43.4, 41.9, 30.9, 28.6, 25.4, 24.1. HRMS (ESI, pos. ion) calcd for $C_{35}H_{39}N_3O_4S$, 598.2724 (M + H); found, 598.2730.

(*R*)-*N*-((*R*)-7-((Dimethylamino)methyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (15). The title compound was prepared according to general procedure A, employing *N*,*N*-dimethylamine, 40% aqueous solution, as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.43 (1H, d, J = 8.8 Hz), 8.23 (1H, s), 8.19 (1H, d, J = 8.4 Hz), 8.03 (1H, d, J = 7.8 Hz), 7.96 (2H, t, J = 6.7 Hz), 7.60−7.69 (3H, m), 7.16 (2H, d, J = 6.7 Hz), 7.00−7.10 (3H, m), 6.58 (1H, s), 6.50 (1H, d, J = 7.8 Hz), 6.15 (1H, d, J = 7.8 Hz), 4.70−4.82 (2H, m), 4.04 (2H, t, J = 5.1 Hz), 3.24 (2H, s), 2.53−2.64 (1H, m), 2.41−2.50 (1H, m), 2.09 (6H, s), 1.80−1.89 (1H, m), 1.61−1.70 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.8, 154.2, 140.4, 138.3, 133.8, 131.4, 129.0, 128.8, 128.5, 128.4, 127.7, 127.6, 127.2, 127.1, 126.9, 122.1, 121.6, 120.4, 116.1, 63.4, 62.8, 55.1, 44.7, 43.4, 41.9, 28.6. HRMS (ESI, pos. ion) calcd for $C_{31}H_{33}N_3O_4S$, 544.2256 (M + H); found, 544.2264. Anal. ($C_{31}H_{33}N_3O_4S\cdot 0.2H_2O$) C, H, N.

(*R*)-3-(Naphthalene-3-sulfonamido)-3-phenyl-*N*-((*R*)-7-(pyrrolidin-1-ylmethyl)chroman-4-yl)propanamide (16). The title compound was prepared according to general procedure A, employing pyrrolidine as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.46 (1H, d, J = 8.6 Hz), 8.18−8.25 (2H, m), 8.02 (1H, d, J = 7.8 Hz), 7.95 (2H, t, J = 7.8 Hz), 7.59−7.69 (3H, m), 7.16 (2H, d, J = 6.7 Hz), 7.00−7.09 (3H, m), 6.66 (1H, br s), 6.56 (1H, d, J = 7.0 Hz), 6.15 (1H, d, J = 7.8 Hz), 4.69−4.82 (2H, m), 3.98−4.10 (2H, m), 3.57 (1H, br s), 3.25−3.43 (2H, m), 2.52−2.64 (2H, m), 2.39−2.48 (2H, m), 1.77−1.89 (1H, m), 1.61−1.77 (5H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.8, 154.2, 140.3, 138.2, 133.7, 131.4, 128.9, 128.7, 128.5, 128.3, 127.6, 127.5, 127.1, 127.0, 126.8, 122.0, 63.4, 55.1, 53.1, 43.3, 41.8, 28.5, 22.8. HRMS (ESI, pos. ion) calcd for $C_{33}H_{35}N_3O_4S$, 570.2418 (M + H); found, 570.2419. Anal. Calcd for $C_{33}H_{35}N_3O_4S\cdot 1.2H_2O$: C, 66.82; H, 6.39; N, 7.08. Found: C, 66.60; H, 5.89; N, 6.90.

(*R*)-*N*-((*R*)-7-(Morpholinomethyl)chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (17). The title compound was prepared according to general procedure A, employing morpholine as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.42 (1H, d, J = 8.6 Hz), 8.22 (1H, s), 8.18 (1H, d, J = 8.4 Hz), 8.02 (1H, d, J = 7.6 Hz), 7.95 (2H, t, J = 7.7 Hz), 7.59−7.68 (3H, m), 7.15 (2H, d, J = 6.7 Hz), 6.99−7.11 (3H, m), 6.59 (1H, s), 6.50 (1H, d, J = 7.6 Hz), 6.16 (1H, d, J = 7.8 Hz), 4.69−4.81 (2H, m), 4.03 (2H, t, J = 5.0 Hz), 3.50−3.57 (4H, m), 3.28 (2H, s), 2.56 (1H, s), 2.45 (1H, s), 2.27 (4H, br s), 1.79−1.88 (1H, m), 1.59−1.69 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.8, 154.2, 140.4, 138.3, 138.2, 133.8, 131.4, 129.0, 128.8, 128.5, 128.4, 127.7, 127.6, 127.2, 127.1, 126.9, 122.1, 121.7, 120.6, 116.2, 66.1, 63.3, 61.8, 55.1, 53.0, 43.4, 41.9, 28.6. HRMS (ESI, pos. ion) calcd for $C_{33}H_{35}N_3O_5S$, 586.2361 (M + H); found, 586.2365. Anal. Calcd for $C_{33}H_{35}N_3O_5S\cdot 0.2H_2O$: C, 67.26; H, 6.05; N, 7.13. Found: C, 67.19; H, 5.88; N, 7.12.

(*R*)-*N*-((*R*)-7-(((2*R*,6*S*)-2,6-Dimethylpiperidin-1-yl)methyl)-chroman-4-yl)-3-(naphthalene-3-sulfonamido)-3-phenylpropanamide (18). The title compound was prepared according to general procedure A, employing (2*R*,6*S*)-2,6-dimethylpiperidine as the amine. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.40 (1H, br s), 8.22 (1H, s), 8.17 (1H, d, J = 8.2 Hz), 8.02 (1H, d, J = 7.8 Hz), 7.95 (2H, t, J = 7.6 Hz), 7.59−7.68 (3H, m), 7.15 (2H, d, J = 7.0 Hz), 6.99−7.08 (3H, m), 6.69 (1H, s), 6.54 (1H, d, J = 8.0 Hz), 6.12 (1H, d, J = 7.8 Hz), 4.74−4.82 (1H, m), 4.70 (1H, q, J = 6.8 Hz), 4.01 (2H, t, J = 4.8 Hz), 3.53 (2H, s), 2.52−2.60 (1H, m), 2.28−2.48 (3H, m), 1.78−1.87 (1H, m), 1.44−1.71 (4H, m), 1.12−1.29 (3H, m), 0.88 (6H, dd, J = 5.7, 3.9 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.7, 154.0, 143.3, 140.4, 138.2, 133.7, 131.4, 128.9, 128.7, 128.3, 128.2, 127.6, 127.5, 127.1, 127.0, 126.8, 126.7, 122.0, 120.3, 118.8, 114.6, 63.1, 57.2, 57.1, 55.0, 53.2, 43.3, 41.8, 34.0, 28.6, 23.6, 21.8. HRMS (ESI, pos. ion) calcd for $C_{36}H_{41}N_3O_4S$, 612.2880 (M + H); found, 612.2888. Anal. Calcd for $C_{36}H_{41}N_3O_4S\cdot 0.2H_2O$: C, 70.26; H, 6.78; N, 6.83. Found: C, 70.17; H, 6.53; N, 6.77.

Compounds **19**−**30** were synthesized by a similar procedure, starting from the corresponding commercially available arylsulfonyl chloride, as noted below, and (*R*)-3-amino-3-phenylpropanoic acid **3**.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(2-chlorophenylsulfonamido)-3-phenylpropanamide (19). The title compound was prepared from 2-chlorophenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.57 (1H, br s), 8.30 (1H, d, J = 8.2 Hz), 7.81 (1H, d, J = 7.4 Hz), 7.47 (1H, t, J = 7.3 Hz), 7.30−7.43 (2H, m), 7.08−7.18 (5H, m), 6.85 (1H, s), 6.72 (1H, d, J = 7.4 Hz), 6.23 (1H, d, J = 7.8 Hz), 4.76−4.84 (1H, m), 4.66−4.75 (1H, m), 4.06−4.18 (2H, m), 3.80 (2H, s), 2.58−2.69 (2H, m), 1.87−1.96 (1H, m), 1.70−1.80 (1H, m), 1.24 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 168.1, 154.3, 139.9,

138.0, 133.5, 131.2, 130.5, 130.4, 128.7, 127.7, 127.2, 127.1, 126.7, 63.7, 55.1, 43.1, 42.0, 28.5. HRMS (ESI, pos. ion) calcd for $C_{29}H_{34}N_3O_4SCl$, 556.2031 (M + H); found, 556.2024.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(3-chlorophenylsulfonamido)-3-phenylpropanamide (20). The title compound was prepared from 3-chlorophenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.31 (1H, d, J = 8.4 Hz), 7.48−7.56 (2H, m), 7.35−7.45 (2H, m), 7.09−7.19 (5H, m), 6.87 (1H, s), 6.74 (1H, d, J = 7.0 Hz), 6.30 (1H, d, J = 7.8 Hz), 4.84 (1H, d, J = 5.7 Hz), 4.63−4.79 (1H, m), 4.08−4.20 (2H, m), 3.84 (2H, s), 2.58 (2H, d, J = 7.2 Hz), 1.84−1.99 (1H, m), 1.63−1.83 (1H, m), 1.26 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 168.0, 154.3, 143.2, 139.8, 133.2, 131.7, 130.6, 128.7, 127.8, 127.2, 126.9, 126.0, 124.8, 66.3, 63.8, 55.4, 43.5, 42.0, 28.6. HRMS (ESI, pos. ion) calcd for $C_{29}H_{34}N_3O_4SCl$, 556.2031 (M + H); found, 556.2031.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(4-chlorophenylsulfonamido)-3-phenylpropanamide (21). The title compound was prepared from 4-chlorophenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.55 (1H, br s), 8.30 (1H, d, J = 7.2 Hz), 7.54 (2H, d, J = 7.4 Hz), 7.42 (2H, d, J = 7.4 Hz), 7.08−7.22 (5H, m), 6.88 (1H, s), 6.75 (1H, s), 6.28 (1H, d, J = 6.3 Hz), 4.78−4.91 (1H, m), 4.65−4.77 (1H, m), 4.07−4.20 (2H, m), 3.84 (2H, s), 2.51−2.62 (2H, m), 1.86−1.96 (1H, m), 1.58−1.79 (1H, m), 1.27 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 168.0, 154.3, 140.2, 140.1, 136.6, 128.7, 128.7, 128.2, 127.8, 127.0, 126.9, 63.7, 55.3, 43.5, 42.0, 33.3, 28.6, 25.2, 24.4. HRMS (ESI, pos. ion) calcd for $C_{29}H_{34}N_3O_4SCl$, 556.2031 (M + H); found, 556.2031. Anal. Calcd for $C_{29}H_{34}N_3O_4SCl\cdot1.8H_2O$: C, 59.18; H, 6.44; N, 7.14. Found: C, 59.14; H, 6.19; N, 7.02.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-phenyl-3-(2-(trifluoromethyl)phenylsulfonamido)propanamide (22). The title compound was prepared from 2-(trifluoromethyl)phenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.59 (1H, br s), 8.31 (1H, d, J = 8.4 Hz), 7.91 (1H, d, J = 7.2 Hz), 7.79 (1H, d, J = 7.2 Hz), 7.58−7.70 (2H, m), 7.12−7.19 (5H, m), 6.86 (1H, s), 6.73 (1H, d, J = 7.0 Hz), 6.29 (1H, d, J = 8.0 Hz), 4.75−4.84 (2H, m), 4.07−4.17 (2H, m), 3.81 (2H, d, J = 0.8 Hz), 2.64 (2H, d, J = 7.4 Hz), 1.87−1.96 (1H, m), 1.70−1.79 (1H, m), 1.25 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 168.1, 154.3, 140.2, 139.7, 132.7, 132.5, 130.7, 129.6, 128.7, 127.8, 127.1, 126.8, 125.7, 125.4, 63.6, 55.2, 43.2, 42.0, 28.5. HRMS (ESI, pos. ion) calcd for $C_{30}H_{34}N_3O_4SF_3$, 590.2294 (M + H); found, 590.2289. Anal. Calcd for $C_{30}H_{34}N_3O_4SF_3\cdot1.8H_2O$: C, 57.92; H, 6.09; N, 6.75. Found: C, 57.85; H, 5.73; N, 6.41.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-phenyl-3-(3-(trifluoromethyl)phenylsulfonamido)propanamide (23). The title compound was prepared from 3-(trifluoromethyl)phenylsulfonyl chloride and acid **3**. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.63 (1H, br s), 8.20−8.27 (1H, m), 7.75−7.85 (2H, m), 7.70 (1H, s), 7.52−7.63 (1H, m), 7.00−7.13 (5H, m), 6.66 (1H, s), 6.53−6.61 (1H, m), 6.18−6.26 (1H, m), 4.71−4.83 (2H, m), 4.00−4.14 (2H, m), 3.49 (2H, s), 2.56 (2H, d, J = 6.0 Hz), 1.82−1.98 (1H, m), 1.62−1.78 (1H, m), 1.04 (9H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.7, 154.3, 142.9, 142.6, 139.6, 130.2, 129.4, 129.0, 128.4, 127.8, 127.0, 126.9, 125.1, 122.9, 122.87, 122.81, 121.50, 120.9, 119.8, 115.3, 63.3, 55.5, 50.1, 45.8, 43.6, 42.0, 28.9. HRMS (ESI, pos. ion) calcd for $C_{30}H_{34}N_3O_4SF_3$, 590.2294 (M + H); found, 590.2291. Anal. Calcd for $C_{30}H_{34}N_3O_4SF_3$: C, 61.11; H, 5.81; N, 7.13. Found: C, 60.94; H, 5.85; N, 7.07.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-phenyl-3-(4-(trifluoromethyl)phenylsulfonamido)propanamide (24). The title compound was prepared from 4-(trifluoromethyl)phenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.74 (1H, br s), 8.32 (1H, d, J = 7.4 Hz), 7.63−7.74 (4H, m), 7.02−7.15 (5H, m), 6.90 (1H, s), 6.71−6.80 (1H, m), 6.31 (1H, d, J = 6.7 Hz), 4.72−4.88 (2H, m), 4.07−4.22 (2H, m), 3.87 (2H, s), 2.59 (2H, d, J = 6.8 Hz), 1.87−1.97 (1H, m), 1.76 (1H, s), 1.29 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.9, 154.3, 145.1, 139.7, 131.6, 131.3, 128.7, 127.8, 127.2, 127.0, 126.9, 125.7, 125.7, 124.8, 122.1, 121.3, 66.3, 63.8, 55.5, 48.5, 43.5, 42.0, 28.5. HRMS (ESI, pos. ion) calcd for $C_{30}H_{34}N_3O_4SF_3$, 590.2294 (M + H); found, 590.2299.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(4-*tert*-butylphenylsulfonamido)-3-phenylpropanamide (25). The title compound was prepared from 4-*tert*-butylphenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.25−8.35 (2H, m), 7.46 (2H, d, J = 8.6 Hz), 7.34 (2H, d, J = 8.6 Hz), 7.06−7.16 (5H, m), 6.87 (1H, s), 6.74 (1H, d, J = 7.6 Hz), 6.24 (1H, d, J = 8.0 Hz), 4.75−4.87 (1H, m), 4.62−4.76 (1H, m), 4.02−4.23 (2H, m), 3.82 (2H, s), 2.52−2.58 (2H, m), 1.85−1.97 (1H, m), 1.68−1.79 (1H, m), 1.19−1.33 (18H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 168.1, 154.6, 154.3, 140.2, 138.4, 128.7, 127.7, 126.9, 126.1, 125.3, 63.7, 55.2, 43.6, 42.0, 34.6, 30.7, 28.6. HRMS (ESI, pos. ion) calcd for $C_{33}H_{43}N_3O_4S$, 578.3047 (M + H); found, 578.3047.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(3,4-dichlorophenylsulfonamido)-3-phenylpropanamide (26). The title compound was prepared from 3,4-dichlorophenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.29 (1H, d, J = 8.2 Hz), 7.66 (1H, d, J = 8.4 Hz), 7.59 (1H, d, J = 2.0 Hz), 7.54 (1H, dd, J = 8.4, 2.2 Hz), 7.13−7.22 (5H, m), 6.73 (1H, s), 6.63 (1H, dd, J = 8.0, 1.0 Hz), 6.29 (1H, d, J = 7.8 Hz), 4.78−4.87 (2H, m), 4.14 (2H, t, J = 5.1 Hz), 3.56 (2H, s), 2.62 (2H, d, J = 7.6 Hz), 1.89−2.04 (1H, m), 1.67−1.84 (1H, m), 1.10 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.7, 154.2, 142.8, 141.6, 139.7, 134.7, 131.3, 130.9, 128.4, 128.2, 127.8, 127.0, 126.9, 126.3, 120.9, 119.7, 115.3, 79.1, 63.3, 55.5, 50.1, 45.8, 43.4, 42.0, 28.8. HRMS (ESI, pos. ion) calcd for $C_{29}H_{33}N_3O_4SCl_2$, 590.1641 (M + H); found, 590.1643. Anal. Calcd for $C_{29}H_{33}N_3O_4SCl_2$: C, 58.98; H, 5.63; N, 7.12. Found: C, 58.85; H, 5.56; N, 7.06.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(2,4-dichlorophenylsulfonamido)-3-phenylpropanamide (27). The title compound was prepared from 2,4-dichlorophenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.69 (1H, d, J = 6.5 Hz), 8.29 (1H, d, J = 8.0 Hz), 7.77 (1H, d, J = 8.4 Hz), 7.54 (1H, s), 7.44 (1H, d, J = 8.2 Hz), 7.09−7.19 (5H, m), 6.86 (1H, s), 6.72 (1H, d, J = 5.7 Hz), 6.28 (1H, d, J = 7.6 Hz), 4.81 (1H, d, J = 5.3 Hz), 4.66−4.73 (1H, m), 4.08−4.19 (2H, m), 3.84 (2H, s), 2.65 (2H, d, J = 7.8 Hz), 1.88−1.98 (1H, m), 1.72−1.81 (1H, m), 1.26 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 168.0, 154.3, 139.8, 137.4, 137.1, 131.9, 131.8, 130.6, 128.7, 128.7, 127.7, 127.3, 127.0, 126.8, 63.7, 55.2, 43.0, 42.0, 28.5. HRMS (ESI, pos. ion) calcd for $C_{29}H_{33}N_3O_4SCl_2$, 590.1641 (M + H); found, 590.1645.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(3-chloro-4-fluorophenylsulfonamido)-3-phenylpropanamide (28). The title compound was prepared from 3-chloro-4-fluorophenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.66 (1H, br s), 8.33 (1H, d, J = 6.7 Hz), 7.44−7.58 (2H, m), 7.38 (1H, t, J = 7.2 Hz), 7.01−7.22 (5H, m), 6.89 (1H, s), 6.76 (1H, d, J = 6.5 Hz), 6.32 (1H, d, J = 6.1 Hz), 4.81−4.92 (1H, m), 4.66−4.80 (1H, m), 4.06−4.27 (2H, m), 3.87 (2H, s), 2.56−2.66 (2H, m), 1.85−2.02 (1H, m), 1.67−1.84 (1H, m), 1.28 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 168.0, 160.0, 157.5, 154.3, 139.6, 138.8, 138.8, 129.0, 128.7, 127.8, 127.6, 127.2, 126.9, 121.3, 119.9, 119.7, 117.3, 117.1, 63.8, 55.6, 43.4, 42.0, 28.6. HRMS (ESI, pos. ion) calcd for $C_{29}H_{33}N_3O_4SClF$, 574.1937 (M + H); found, 574.1945.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(3-chloro-2-methylphenylsulfonamido)-3-phenylpropanamide (29). The title compound was prepared from 3-chloro-2-methylphenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.63 (1H, d, J = 8.4 Hz), 8.30 (1H, d, J = 8.4 Hz), 7.73 (1H, d, J = 8.0 Hz), 7.54 (1H, d, J = 8.0 Hz), 7.25 (1H, t, J = 7.9 Hz), 7.09−7.20 (3H, m), 7.03−7.09 (2H, m), 6.87 (1H, s), 6.72 (1H, d, J = 5.3 Hz), 6.22 (1H, d, J = 7.8 Hz), 4.77−4.84 (1H, m), 4.58−4.65 (1H, m), 4.08−4.19 (2H, m), 3.86 (2H, s), 2.63 (2H, d, J = 7.6 Hz), 2.41 (3H, s), 1.87−1.98 (1H, m), 1.71−1.80 (1H, m), 1.27 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 168.0, 154.3, 141.1, 140.0, 135.4, 133.9, 132.7, 128.7, 127.8, 127.6, 127.1, 126.9, 126.6,

63.8, 55.0, 43.3, 42.0, 28.5, 16.4. HRMS (ESI, pos. ion) calcd for $C_{30}H_{36}N_3O_4SCl$, 570.2187 (M + H); found, 570.2185. Anal. Calcd for $C_{30}H_{36}N_3O_4SCl \cdot 2.0H_2O$: C, 59.44; H, 6.65; N, 6.93. Found: C, 59.26; H, 6.23; N, 6.79.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(5-chloro-2,4-difluorophenylsulfonamido)-3-phenylpropanamide (30). The title compound was prepared from 5-chloro-2,4-difluorophenylsulfonyl chloride and acid **3**. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.95 (1H, br s), 8.33 (1H, d, J = 8.2 Hz), 7.55 (1H, t, J = 7.5 Hz), 7.50 (1H, t, J = 9.6 Hz), 7.15 (5H, s), 6.91 (1H, s), 6.77 (1H, d, J = 7.6 Hz), 6.32 (1H, d, J = 8.0 Hz), 4.80−4.95 (1H, m), 4.66−4.79 (1H, m), 4.10−4.23 (2H, m), 3.91 (2H, s), 2.60−2.72 (2H, m), 1.90−2.00 (1H, m), 1.73−1.83 (1H, m), 1.31 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.9, 154.3, 139.4, 136.8, 130.6, 128.8, 127.8, 127.2, 126.8, 126.5, 63.9, 55.4, 44.1, 43.1, 42.1, 28.5, 25.3. HRMS (ESI, pos. ion) calcd for $C_{29}H_{32}N_3O_4SClF_2$, 592.1842 (M + H); found, 592.1835.

Compounds **31**−**40** were prepared by a similar procedure, employing 3-(trifluoromethyl)phenylsulfonyl chloride or 3,4-dichlorophenylsulfonyl chloride in reaction with (*R*)-3-amino-3-(4-fluorophenyl)-propanoic acid (for compounds **31** and **33**−**40**) or (*R*)-3-amino-3-(4-cyanophenyl)-propanoic acid (for compound **32**) as the first step. Final coupling with each corresponding amine was accomplished using general procedure A.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(4-fluorophenyl)-3-(3-(trifluoromethyl)phenylsulfonamido)-propanamide (31). The title compound was prepared from 3-(trifluoromethyl)phenylsulfonyl chloride in the first step, employing *tert*-butylamine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.30 (1H, d, J = 8.4 Hz), 7.83 (2H, t, J = 7.6 Hz), 7.60−7.64 (2H, m), 7.09 (2H, dd, J = 8.5, 5.6 Hz), 6.85 (2H, t, J = 8.9 Hz), 6.79 (1H, s), 6.65 (1H, d, J = 7.6 Hz), 6.31 (1H, d, J = 7.8 Hz), 4.72−4.93 (2H, m), 4.03−4.23 (2H, m), 3.71 (2H, s), 2.53−2.66 (2H, m), 1.86−2.01 (2H, m), 1.70−1.78 (1H, m), 1.17 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.7, 162.4, 160.0, 154.3, 142.4, 135.7, 130.2, 129.3, 128.9, 128.9, 128.5, 122.8, 121.9, 114.6, 114.4, 63.6, 54.9, 43.5, 42.0, 28.7, 27.1, 21.1. HRMS (ESI, pos. ion) calcd for $C_{30}H_{33}F_4N_3O_4S$, 608.2192 (M + H); found, 608.2192.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(4-cyanophenyl)-3-(3-(trifluoromethyl)phenylsulfonamido)-propanamide (32). The title compound was prepared from 3-(trifluoromethyl)phenylsulfonyl chloride in the first step, employing *tert*-butylamine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.34 (1H, d, J = 8.4 Hz), 7.85 (2H, t, J = 9.0 Hz), 7.60−7.67 (2H, m), 7.53 (2H, d, J = 8.2 Hz), 7.26 (2H, d, J = 8.2 Hz), 6.78 (1H, s), 6.67 (1H, d, J = 8.0 Hz), 6.31 (1H, d, J = 7.2 Hz), 4.79−4.87 (2H, m), 4.12 (2H, t, J = 5.1 Hz), 3.69 (2H, s), 2.55−2.67 (2H, m), 1.89−1.96 (2H, m), 1.69−1.78 (1H, m), 1.16 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.4, 154.3, 145.0, 142.2, 131.8, 130.4, 130.3, 129.3, 129.0, 128.6, 128.5, 128.0, 124.5, 122.8, 121.8, 120.2, 118.4, 110.0, 63.4, 55.2, 43.0, 42.0, 28.6, 27.3, 21.1. HRMS (ESI, pos. ion) calcd for $C_{31}H_{33}F_3N_4O_4S$, 615.2239 (M + H); found, 615.2235. Anal. Calcd for $C_{31}H_{33}F_3N_4O_4S \cdot 1.2H_2O$: C, 58.52; H, 5.61; N, 8.80. Found: C, 58.27; H, 5.41; N, 8.58.

(*R*)-3-(4-Fluorophenyl)-*N*-((*R*)-7-((2-methoxyethylamino)methyl)chroman-4-yl)-3-(3-(trifluoromethyl)phenylsulfonamido)-propanamide (33). The title compound was prepared from 3-(trifluoromethyl)phenylsulfonyl chloride in the first step, employing 2-methoxyethylamine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.25 (1H, d, J = 8.4 Hz), 7.83 (2H, t, J = 7.3 Hz), 7.60−7.65 (2H, m), 7.08 (2H, dd, J = 8.5, 5.6 Hz), 6.85 (2H, t, J = 8.8 Hz), 6.64 (1H, s), 6.54 (1H, dd, J = 7.9, 1.1 Hz), 6.19 (1H, d, J = 7.8 Hz), 4.75−4.83 (2H, m), 4.09 (2H, t, J = 5.0 Hz), 3.55 (2H, s), 3.35 (2H, t, J = 5.7 Hz), 3.22 (3H, s), 3.17 (1H, d, J = 2.9 Hz), 2.52−2.61 (4H, m), 1.91 (1H, ddd, J = 8.6, 5.3, 5.1 Hz), 1.66−1.76 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.5, 159.9, 154.3, 142.3, 141.3, 135.6, 130.2, 129.3, 128.9, 128.8, 128.3, 124.5, 122.7, 121.8, 121.1, 119.4, 115.2, 114.5, 114.3, 71.5, 63.3, 57.9, 54.8, 52.2, 47.5, 43.4, 41.9, 28.7. HRMS (ESI, pos. ion) calcd for $C_{29}H_{31}F_4N_3O_5S$, 610.1985 (M + H); found, 610.1986. Anal. Calcd for $C_{29}H_{31}F_4N_3O_5S$: C, 57.13; H, 5.13; N, 6.89. Found: C, 57.32; H, 5.19; N, 6.91.

(*R*)-3-(4-Fluorophenyl)-*N*-((*R*)-7-((isopropylamino)methyl)-chroman-4-yl)-3-(3-(trifluoromethyl)phenylsulfonamido)-propanamide (34). The title compound was prepared from 3-(trifluoromethyl)phenylsulfonyl chloride in the first step, employing isopropylamine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.26 (1H, d, J = 8.4 Hz), 7.83 (2H, t, J = 7.1 Hz), 7.60−7.65 (2H, m), 7.07 (2H, dd, J = 8.5, 5.6 Hz), 6.85 (2H, t, J = 8.7 Hz), 6.66 (1H, s), 6.55 (1H, d, J = 7.8 Hz), 6.18 (1H, d, J = 7.8 Hz), 4.79 (2H, q, J = 8.3 Hz), 4.09 (2H, t, J = 5.1 Hz), 3.53 (2H, s), 2.53−2.71 (3H, m), 1.91 (1H, td, J = 9.5, 5.2 Hz), 1.66−1.76 (1H, m), 0.94 (6H, d, J = 6.1 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.6, 162.4, 160.0, 154.3, 142.4, 141.9, 135.6, 130.2, 129.0, 128.9, 128.3, 122.8, 121.0, 119.5, 115.3, 114.6, 114.4, 63.3, 54.8, 49.7, 46.7, 43.5, 41.9, 28.8, 22.6. HRMS (ESI, pos. ion) calcd for $C_{29}H_{31}F_4N_3O_4S$, 594.2036 (M + H); found, 594.2037. Anal. Calcd for $C_{29}H_{31}F_4N_3O_4S$: C, 58.67; H, 5.26; N, 7.08. Found: C, 58.44; H, 5.20; N, 7.10.

(*R*)-3-(4-Fluorophenyl)-*N*-((*R*)-7-((isobutylamino)methyl)chroman-4-yl)-3-(3-(trifluoromethyl)phenylsulfonamido)-propanamide (35). The title compound was prepared from 3-(trifluoromethyl)phenylsulfonyl chloride in the first step, employing isobutylamine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.29 (1H, d, J = 7.8 Hz), 7.79−7.88 (2H, m), 7.57−7.68 (2H, m), 7.03−7.13 (2H, m), 6.85 (2H, t, J = 8.1 Hz), 6.70 (1H, s), 6.57 (1H, d, J = 7.0 Hz), 6.20 (1H, d, J = 6.8 Hz), 4.73−4.86 (2H, m), 4.11 (2H, s), 3.62 (2H, s), 2.54−2.69 (2H, m), 2.29 (2H, d, J = 4.9 Hz), 1.87−1.97 (1H, m), 1.63−1.78 (2H, m), 0.85 (6H, d, J = 6.1 Hz). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.6, 160.0, 154.3, 142.4, 135.6, 130.2, 129.0, 128.9, 128.4, 124.6, 122.8, 119.8, 115.7, 114.6, 114.4, 63.4, 55.9, 54.9, 43.5, 41.9, 28.7, 27.4, 20.5. HRMS (ESI, pos. ion) calcd for $C_{30}H_{33}F_4N_3O_4S$, 608.2189 (M + H); found, 608.2189.

(*R*)-*N*-((*R*)-7-(((Cyclobutylamino)methyl)chroman-4-yl)-3-(4-fluorophenyl)-3-(3-(trifluoromethyl)phenylsulfonamido)-propanamide (36). The title compound was prepared from 3-(trifluoromethyl)phenylsulfonyl chloride in the first step, employing cyclobutylamine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.27 (1H, d, J = 8.4 Hz), 7.83 (2H, t, J = 7.6 Hz), 7.60−7.65 (2H, m), 7.08 (2H, dd, J = 8.5, 5.6 Hz), 6.85 (2H, t, J = 8.9 Hz), 6.67 (1H, s), 6.56 (1H, d, J = 7.8 Hz), 6.22 (1H, d, J = 7.8 Hz), 4.75−4.84 (2H, m), 4.06−4.14 (2H, m), 3.52 (2H, s), 3.10−3.18 (1H, m, J = 7.6, 7.6, 7.6, 7.6 Hz), 2.53−2.64 (2H, m), 1.97−2.11 (2H, m), 1.85−1.96 (2H, m), 1.66−1.79 (3H, m), 1.45−1.68 (2H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.6, 162.4, 160.0, 154.3, 142.4, 135.7, 135.6, 130.2, 129.0, 128.9, 128.4, 124.6, 122.8, 122.8, 121.6, 119.9, 115.7, 114.6, 114.4, 63.4, 54.8, 52.4, 49.0, 43.5, 42.0, 29.4, 28.7, 21.0, 14.4. HRMS (ESI, pos. ion) calcd for $C_{30}H_{31}F_4N_3O_4S$, 606.2036 (M + H); found, 606.2037. Anal. Calcd for $C_{30}H_{31}F_4N_3O_4S \cdot 1.3H_2O$: C, 57.28; H, 5.38; N, 6.68. Found: C, 57.03; H, 5.16; N, 6.66.

(*R*)-3-(4-Fluorophenyl)-*N*-((*R*)-7-(pyrrolidin-1-ylmethyl)chroman-4-yl)-3-(3-(trifluoromethyl)phenylsulfonamido)-propanamide (37). The title compound was prepared from 3-(trifluoromethyl)phenylsulfonyl chloride in the first step, employing pyrrolidine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.68 (1H, d, J = 9.0 Hz), 8.28 (1H, d, J = 8.4 Hz), 7.84 (2H, t, J = 7.1 Hz), 7.60−7.65 (2H, m), 7.08 (2H, dd, J = 8.4, 5.7 Hz), 6.85 (2H, t, J = 8.8 Hz), 6.62 (1H, s), 6.54 (1H, d, J = 7.8 Hz), 6.19 (1H, d, J = 7.8 Hz), 4.74−4.84 (2H, m), 4.10 (2H, t, J = 5.0 Hz), 3.39−3.53 (2H, m), 2.54−2.64 (2H, m), 2.39 (4H, s), 1.88−1.96 (1H, m), 1.64−1.76 (5H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 165.1, 159.9, 157.5, 151.8, 139.9, 133.1, 133.1, 127.8, 126.8, 126.5, 126.4, 126.0, 125.9, 120.4, 120.3, 119.4, 117.6, 112.1, 111.9, 60.9, 52.4, 50.8, 41.0, 39.4, 26.2, 20.5. HRMS (ESI, pos. ion) calcd for $C_{30}H_{31}F_4N_3O_4S$, 606.2036 (M + H); found, 606.2039. Anal. Calcd for $C_{30}H_{31}F_4N_3O_4S$: C, 59.49; H, 5.16; N, 6.94. Found: C, 59.38; H, 5.22; N, 6.92.

(*R*)-3-(4-Fluorophenyl)-*N*-((*R*)-7-(piperidin-1-ylmethyl)chroman-4-yl)-3-(3-(trifluoromethyl)phenylsulfonamido)-propanamide (38). The title compound was prepared from 3-(trifluoromethyl)phenylsulfonyl chloride in the first step, employing piperidine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.67 (1H, d, J = 9.0 Hz), 8.26 (1H, d, J = 8.6 Hz), 7.83 (2H, t, J = 6.9 Hz), 7.59−7.65 (2H, m), 7.07 (2H, dd, J = 8.1, 5.6 Hz), 6.85 (2H, t, J = 8.8 Hz), 6.59 (1H, s), 6.52 (1H, d, J = 7.8 Hz), 6.17 (1H, d, J = 7.8 Hz), 4.74−4.83 (2H, m), 4.09 (2H, t, J = 5.0 Hz), 3.26 (2H, s), 2.53−2.67 (2H, m), 2.24 (4H, s), 1.87−1.96 (1H, m), 1.66−1.76 (1H, m), 1.41−1.50 (4H, m), 1.37 (2H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.5, 162.4, 159.9, 154.2, 142.3, 139.1, 135.5, 130.2, 128.9, 128.8, 128.3, 124.5, 122.7, 121.8, 121.4, 120.2, 116.0, 114.5, 114.3, 63.3, 54.8, 53.6, 43.4, 41.8, 28.6, 25.4. MS (ESI, pos. ion): *m/z* 620.2 (M + H). Anal. Calcd for $C_{31}H_{33}F_4N_3O_4S$: C, 60.09; H, 5.37; N, 6.78. Found: C, 59.95; H, 5.40; N, 6.82.

(*R*)-*N*-((*R*)-7-((*tert*-Butylamino)methyl)chroman-4-yl)-3-(3,4-dichlorophenylsulfonamido)-3-(4-fluorophenyl)propanamide (39). The title compound was prepared from 3,4-dichlorophenylsulfonyl chloride in the first step, employing *tert*-butylamine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.30 (1H, d, J = 8.4 Hz), 7.64 (1H, d, J = 9.0 Hz), 7.47−7.51 (2H, m), 7.12 (2H, dd, J = 8.2, 5.7 Hz), 6.94 (2H, t, J = 8.8 Hz), 6.76 (1H, s), 6.64 (1H, d, J = 7.6 Hz), 6.27 (1H, d, J = 7.8 Hz), 4.83 (1H, dd, J = 13.9, 7.4 Hz), 4.75 (1H, t, J = 7.8 Hz), 4.08−4.15 (2H, m), 3.66 (2H, s), 2.53−2.63 (2H, m), 1.88−1.96 (2H, m), 1.65−1.81 (1H, m), 1.14 (9H, s). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.6, 162.4, 160.0, 154.2, 141.3, 135.7, 134.7, 131.3, 130.9, 129.0, 128.9, 128.3, 128.1, 126.3, 114.5, 114.3, 54.8, 45.1, 43.2, 41.9, 28.6, 27.4, 21.0. HRMS (ESI, pos. ion) calcd for $C_{29}H_{32}Cl_2FN_3O_4S$, 608.1536 (M + H); found, 608.1536.

(*R*)-3-(3,4-Dichlorophenylsulfonamido)-3-(4-fluorophenyl)-*N*-((*R*)-7-((2-methoxyethylamino)methyl)chroman-4-yl)propanamide (40). The title compound was prepared from 3,4-dichlorophenylsulfonyl chloride in the first step, employing 2-methoxyethylamine as the amine in the final step. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.27 (1H, d, J = 8.4 Hz), 7.64 (1H, d, J = 8.2 Hz), 7.47−7.51 (2H, m), 7.12 (2H, dd, J = 8.5, 5.6 Hz), 6.94 (2H, t, J = 8.8 Hz), 6.65 (1H, s), 6.55 (1H, d, J = 8.0 Hz), 6.19 (1H, d, J = 8.0 Hz), 4.72−4.83 (2H, m), 4.09 (2H, t, J = 5.1 Hz), 3.56 (2H, s), 3.36 (2H, t, J = 5.7 Hz), 3.22 (3H, s), 3.17 (1H, d, J = 3.7 Hz), 2.53−2.62 (4H, m), 1.87−1.96 (1H, m), 1.67−1.76 (1H, m). ^{13}C NMR (101 MHz, DMSO-d_6) δ ppm 167.5, 154.3, 141.3, 141.2, 135.7, 134.7, 131.3, 130.9, 129.0, 128.9, 128.3, 128.2, 126.3, 121.1, 119.5, 115.2, 114.5, 114.3, 71.4, 63.3, 57.9, 54.8, 52.1, 47.4, 43.3, 41.9, 28.7. HRMS (ESI, pos. ion) calcd for $C_{28}H_{30}Cl_2FN_3O_5S$, 610.1333 (M + H); found, 610.1315. Anal. Calcd for $C_{28}H_{30}Cl_2FN_3O_5S$: C, 55.08; H, 4.95; N, 6.88. Found: C, 55.28; H, 5.00; N, 6.96.

In Vitro Assays. The binding and cellular functional B1 assays have been described in detail previously. See reference 12.

Pharmacokinetic Studies. Male Sprague−Dawley rats (weight range 225−280 g) with surgically implanted femoral vein and jugular vein cannulate were obtained from Hilltop Lab Animals Inc. (Scottsdale, PA) or Charles River Laboratories, Inc. (Wilmington, MA). Animals were fasted overnight and, on the following day, compounds were administered either by oral gavage or by intravenous bolus injection. Oral formulations were prepared 24−48 h prior to dosing, while intravenous formulations were prepared on the day of dosing. Blood samples were collected over 6 or 8 h via jugular cannula into a heparinized tube. Following centrifugation, plasma samples were stored in a freezer to maintain −70 °C until analysis. Lithium-heparinized plasma samples (40 μL) were precipitated with acetonitrile by reverse phase (C-18) LC-MS/MS with API3000 triple quadrupole mass spectromter operated in the positive multiple reaction monitoring mode. Study sample concentrations were determined from a weighted ($1/\chi^2$) linear regression of peak area ratios (analyte peak area/IS peak area) versus the theoretical concentrations of the calibration standards. Pharmacokinetic parameters were calculated by noncompartmental methods using Watson SMDA (Thermo: Version 6.4.0.02)

In Vivo Pharmacology. Rabbit Blood Pressure Model. In the pharmacodynamic model, the ability of compound **38** to antagonize a B1 agonist DAK[10]-induced blood pressure response was measured. Five hours prior to blood pressure measurements, New Zealand white rabbits (2.0−3.0 kg, n = 1/dose group) were given a sublethal bolus injection of LPS (25 μg/kg, i.v.) to induce functional B1 receptors. Animals were anesthetized and underwent a dual carotid artery catheterization to measure mean arterial blood pressure from the left carotid artery and to inject agonist (DAK) or obtain blood samples from the right carotid artery. The baseline B1 receptor-mediated response was established using two separate bolus EC_{80} injections of the B1 receptor agonist (1 μg; i.v.) in the right carotid artery catheter. A s.c. injection of compound **38** (1, 2, 4, 10, or 20 mpk) was then given. At 30 and 75 min post-compound **38**, blood samples were collected for PK for determination of the plasma concentration of the antagonist and an agonist-induced cardiovascular response was measured. Therefore, for each dose, two data points were generated for determining the dose−response effect of compound **38**.

Rabbit Inflammatory Pain Model. In the efficacy model, the ability of compound **38** to reverse inflammation-associated hyperalgesia in the rabbit was evaluated.[20] Male New Zealand white rabbits (500−700 g) were weighed, and the plantar surface of their right hind paw was shaved. Each rabbit was placed into an individual Plexiglas enclosure with a mesh floor and allowed to acclimate to the enclosure for 30 min. Baseline punctate withdrawal thresholds (*g*) then were determined for each animal using an electronic von Frey (Electrovonfrey) apparatus applied to the plantar surface of the right hind paw. Three withdrawal readings were taken from the right hind paw of each rabbit, and the mean of the three readings was used as the rabbit's baseline withdrawal threshold. Each rabbit then received an injection of lambda carrageenan (3% in physiological saline, 200 μL injection volume) under the plantar surface of the right hind paw. Compound **38** (0, 1, 3, 10 mg/kg; 1 mL/kg in 20% Captisol) or DALK[9,10] (peptide antagonist for the BK B1 receptor; 10 mg/kg; 1 mL/kg in PBS) was administered s.c. at various doses post-carrageenan (2 h and 2.5 h post-carrageenan, respectively). Following injection of the test compound, each rabbit was returned to its Plexiglas enclosure. At 3 h post-carrageenan injection, three more mechanical withdrawal readings were obtained from the rabbit, and the mean of these three readings was used as the rabbit's post-carrageenan punctate mechanical withdrawal threshold (*g*).

The mean pre-carrageenan withdrawal threshold was subtracted from the mean post-carrageenan withdrawal threshold to provide a threshold "difference score" for each rabbit. (In the absence of drug treatment, carrageenan typically results in "difference scores" of −30 to −40 g, indicating the presence of robust punctate mechanical hyperalgesia.) Each difference score, in turn, was converted to value indicating percentage of maximum possible effect (% MPE) using the following formula

$$\% \text{ MPE} = (E - E_{\text{min}}) \times 100/(E_{\text{max}} - E_{\text{min}})$$

The mean "difference score" for rabbits in the vehicle control group was used as E_{min}, and E_{max} was defined as a difference score of 0 (i.e., the absence of mechanical hyperalgesia).

Compound 38. A one-factor ANOVA performed on % MPE values revealed a significant main effect of compound A dose ($F(3,28) = 4.926$; $P < 0.01$). Post-hoc Dunnett's tests revealed that 10 mg/kg, s.c. resulted in significantly higher % MPE values compared with vehicle ($P < 0.01$). The dose of 10 mg/kg resulted in a mean % MPE value of 45.5%.

DALK. DALK administered at 10 mg/kg, s.c. resulted in significantly higher % MPE values compared with its vehicle ($P < 0.01$). This dose resulted in a mean % MPE value of 78.0%.

Supporting Information Available: Purity data for all new compounds (combustion analysis or HPLC). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Marceau, F.; Regoli, D. Bradykinin Receptor Ligands: Therapeutic Perspectives. *Nat. Rev. Drug Discovery* **2004**, *3*, 845−852.

(2) Calixto, J. B.; Cabrini, D. A.; Ferreira, J.; Campos, M. M. Inflammatory Pain: Kinins and Antagonists. *Curr. Opin. Anaesthesiol.* **2001**, *14*, 519−526.

(3) (a) Calixto, J. B.; Medeiros, R.; Fernandes, E. S.; Ferreira, J.; Cabrini, D. A.; Campos, M. M. Kinin B1 Receptors: Key G-Protein-Coupled Receptors and Their Role in Inflammatory and Painful Processes. *Br. J. Pharmacol.* **2004**, *143*, 803−818. (b) Dray, A. Kinins and Their Receptors in Hyperalgesia. *Can. J. Physiol. Pharmacol.* **1997**, *75*, 704−712.

(4) Marceau, F.; Bachvarov, D. R. Kinin Receptors. *Clin. Rev. Allergy Immunol.* **1998**, *16*, 385−401.

(5) Leeb Lundberg, L. M. F.; Marceau, F.; Mueller-Esterl, W.; Pettibone, D. J.; Zuraw, B. L. International Union of Pharmacology. XLV. Classification of the Kinin Receptor Family: From Molecular Mechanisms to Pathophysiological Consequences. *Pharmacol. Rev.* **2005**, *57*, 27−77.

(6) Ahluwalia, A.; Perretti, M. B1 Receptors as a New Inflammatory Target. Could This B the 1? *Trends Pharmacol. Sci.* **1999**, *20*, 100−104.

(7) (a) Pesquero, J. B.; Araujo, R. C.; Heppenstall, P. A.; Stucky, C. L.; Silva, J. A., Jr.; Walther, T.; Oliviera, S. M.; Pesquero, J. L.; Paiva, A. C. M.; Calixton, J. B.; Lewin, G. R.; Bader, M. Hypoalgesia and Altered Inflammatory Responses in Mice Lacking Kinin B1 Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 8140−8145. (b) Ferreira, J.; Campos, M. M.; Pesquero, J. B.; Araujo, R. C.; Bader, M.; Calixto, J. B. Evidence for the Participation of Kinins in Freund's Adjuvant-Induced Inflammatory and Nociceptive Responses in Kinin B1 and B2 Knockout Mice. *Neuropharmacology* **2001**, *41*, 1006−1012. (c) Ferreira, J.; Beirith, A.; Mori, M. A. S.; Araujo, R. C.; Bader, M.; Pesquero, J. B.; Calixto, J. B. Reduced Nerve Injury-Induced Neuropathic Pain in Kinin B1 Receptor Knock-Out Mice. *J. Neurosci.* **2005**, *25*, 2405−2412. (d) Gabra, B. H.; Merino, V. F.; Bader, M.; Pesquero, J. B.; Sirois, P. Absence of Diabetic Hyperalgesia in Bradykinin B1 Receptor-Knockout Mice. *Regul. Pept.* **2005**, *127*, 245−248.

(8) (a) Borkowski, J. A.; Ransom, R. W.; Seabrook, G. R.; Trumbauer, M.; Chen, H.; Hill, R. G.; Strader, C. D.; Hess, J. F. Targeted Disruption of a B2 Bradykinin Receptor Gene in Mice Eliminated Bradykinin Action in Smooth Muscle and Neurons *J. Biol. Chem.* **1995**, *270*, 13706−13710. (b) Alfie, M. E.; Sigmon, D. H.; Pomposiello, S. I.; Carretero, O. A. Effect of High Salt Intake in Mutant Mice Lacking Brakykinin B2 Receptors. *Hypertension* **1997**, *29*, 483−487.

(9) (a) For a review of the discovery of peptide bradykinin antaonists, see: Stewart, J. M. Bradykinin Antagonists: Discovery and Development. *Peptides* **2004**, *25*, 527−532 and references therein. (b) Dray, A.; Perkins, M. N. Bradykinin and Inflammatory Pain. *Trends Neurosci.* **1993**, *16*, 99−104. (c) Perkins, M. N.; Campbell, E.; Dray, N. Antinociceptive Activity of the Bradykinin B1 and 2 Receptor Antagonists, des-Arg9-[Leu8]-BK and HOE 140, in Two Models of Persistent Hyperalgesia in the Rat. *Pain* **1993**, *53*, 191−197. (d) Perkins, M. N.; Kelly, D. Induction of Bradykinin B1 Receptors In Vivo in a Model of Ultraviolet Irradiation Induced Hyperalgesia in the Rat. *Can. J. Physiol. Pharmacol.* **1993**, *110*, 1441−1444. (e) Davis, A. J.; Perkins, M. N.; Substance P and Capsaicin-Induced Mechanical Hyperalgesia in the Rat Knee Joint: The Involvement of Bradykinin B1 and B2 Receptors. *Br. J. Pharmacol.* **1996**, *118*, 2206−2212.

(10) (a) Gabra, B. H.; Sirois, P. Kinin B1 Receptor Antagonists Inhibit Diabetes-Induced Hyperalgesia in Mice. *Neuropeptides* **2003**, *37*, 36−44. (b) Mason, G. S.; Cumberbatch, M. J.; Hill, R. G.; Rupniak, N. M. J. The Bradykinin B1 Receptor Antagonist B9858 Inhibits a Nociceptive Spinal Reflex in Rabbits. *Can. J. Physiol. Pharmacol.* **2002**, *80*, 264−268. (c) Gabra, B. H.; Sirois, P. Beneficial Effect of Chronic Treatment with the Selective Bradykinin B1 Receptor Antagonists, R-715 and R-954, in Attenuating Streptozotocin-Diabetic Thermal Hyperalgesia in Mice. *Peptides* **2003**, *24*, 1131−1139. (d) Rashid, M. H.; Inoue, M.; Matsumoto, M.; Ueda, H. Switching of Bradykinin-Mediated Nociception Following Partial Sciatic Nerve Injury in Mice. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 1158−1164. (e) Ferreira, J.; Beirith, A.; Mori, M. A. S.; Araujo, R. C.; Bader, M.; Pesquero, J. B.; Calixto, J. B. Reduced Nerve Injury-Induced Neuropathic Pain in Kinin B1 Receptor Knock-Out Mice. *J. Neurosci.* **2005**, *25*, 2405−2412. (f) Levy, D.; Zochodne, D. W. Increased mRNA Expression of the B1 and B2 Bradykinin Receptors and Antinociceptive Effects of their Antagonists in an Animal Model of Neuropathic Pain. *Pain* **2000**, *86*, 265−271. (g) Yamaguchi-Sase, S.; Hayashi, I.; Okamoto, H.; Nara, Y.; Matsuzaki, S.; Hoka, S.; Majima, M. Amelioration of Hyperalgesia by Kinin Receptor Antagonists or Kininogen Deficiency in Chronic Constriction Nerve Injury in Rats. *Inflammation Res.* **2003**, *52*, 164−169. (h) Gabra, B. H.; Merino, V. F.; Bader, M.; Pesquero, J. B.; Sirois, P. Absence of Diabetic Hyperalgesia in Bradykinin B1 Receptor-Knockout Mice. *Regul. Pept.* **2005**, *127*, 245−248.

(11) (a) See: Bock, M. G.; Hess, J. F.; Pettibone, D. J. Bradykinin-1 Receptor Antagonists. *Annu. Rep. Med. Chem.* **2003**, *38*, 111−120 and references cited therein. (b) Kuduk, S. D.; Ng, C.; Fenf, D.-M.; Wai, J. M.-C.; Chang, R. S. L.; Harrell, C. M.; Murphy, K. L.; Ransom, R. W.; Reiss, D.; Ivarsson, M.; Mason, G.; Boyce, S.; Tang, C.; Prueksaritanont, T.; Freidinger, R. M.; Pettibone, D. J.; Bock, M. G. 2,3-Diaminopyridine Bradykinin B1 Antagonists. *J. Med. Chem.* **2004**, *47*, 6439−6442. (c) Ritchie, T. J.; Dziadulewicz, E. K.; Culshaw, A. J.; Muller, W.; Burgess, G. M.; Bloomfield, G. C.; Drake, G. S.; Dunstan, A. R.; Beattie, D.; Hughes, G. A.; Ganju, P.; McIntyre, P.; Bevan, S. J.; Davis, C.; Yaqoob, M. Potent and Orally Bioavailable Non-Peptide Antagonists at the Human Bradykinin B1 Receptor Based on a 2-Alkylamino-5-sulfamoylbenzamide Core. *J. Med. Chem.* **2004**, *47*, 4642−4644. (d) Gougat, J.; Ferrari, B.; Sarran, L.; Planchenault, C.; Poncelet, M.; Maruani, J.; Alonso, R.; Cudennec, A.; Croci, T.; Guagnini, F.; Urban-Szabo, K.; Martinolle, J.-P.; Soubrie, P.; Finance, O.; Le Fur, G. SSR240612 [(2*R*)-2-[((3*R*)-3-(1,3-Benzodioxol-5-yl)-3-{[(6-methoxy-2-naphthyl)sulfonyl]amino}-propanoyl)amino]3-(4-{[2*R*, 6*S*)-2,6-dimethylpiperidinyl]methyl}-phenyl)-*N*-isopropyl-*N*-methylpropanamide Hydrochloride], a New Nonpeptide Antagonist of the Bradykinin B1 Receptor: Biochemical and Pharmacological Characterization. *J. Pharmacol. Exp. Ther.* **2004**, *309*, 661−669. (e) Conley, R. K.; Wheeldon, A.; Webb, J. K.; DiPardo, R. M.; Homnick, C. F.; Bock, M. G.; Chen, T.-B.; Chang, R. S. L.; Pettibone, D. J.; Boyce, S. Inhibition of Acute Nociceptive Responses in Rat Spinal Cord by a Bradykinin B1 Antagonist. *Eur. J. Pharmacol.* **2005**, *527*, 44−51. (f) Wood, M. R.; Schirripa, K. M.; Kim, J. J.; Wan, B.-L.; Murphy, K. L.; Ransom, R. W.; Chang, R. S. L.; Tang, C.; Prueksaritanont, T.; Detwiler, T. J.; Hettrick, L. A.; Landis, E. R.; Leonard, Y. M.; Krueger, J. A.; Lewis, S. D.; Pettibone, D. J.; Freidinger, R. M.; Bock, M. B. Cyclopropylamino Acid Amide as a Pharmacophoric Replacement for 2,3-Diaminopyridine. Application to the Design of a Novel Bradykinin B1 Receptor Antagonist. *J. Med. Chem.* **2006**, *49*, 1231−1234. (g) Kuduk, S. D.; DiMarco, C. N.; Chang, R. K.; Wood, M. R.; Kim, J. J.; Schirripa, K. M.; Murphy, K. L.; Ransom, R. W.; Tang, C.; Torrent, M.; Ha, S.; Prueksaritanont, T.; Pettibone, D. J.; Bock, M. G. 5-Piperazinyl Pyridine Carboxamide Bradykinin B1 Antagonists. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2791−2795. (h) Kuduk, S. D.; DiMarco, C. N.; Chang, R. K.; Wood, M. R.; Schirripa, K. M.; Kim, J. J.; Wai, J. M. C.; DiPardo, R. M.; Murphy, K. L.; Ransom, R. W.; Harrell, C. M.; Reiss, D. R.; Holahan, M. A.; Cook, J.; Hess, J. F.; Sain, N.; Urban, M. O.; Tang, C.; Prueksaritanont, T.; Pettibone, D. J.; Bock, M. G. Development of Orally Bioavailable and CNS Penetrant Biphenylaminocyclopropane Carboxamide Bradykinin B1 Receptor Antagonists. *J. Med. Chem.* **2007**, *50*, 272−282.

(12) D'Amico, D. C.; Aya, T.; Human, J.; Fotsch, C.; Chen, J. J.; Biswas, K.; Riahi, B.; Norman, M. H.; Willoughby, C. A.; Hungate, R.; Reider, P. J.; Biddlecome, G.; Lester-Ziener, D.; Van Staden, C.; Johnson, E.; Kamassah, A.; Arik, L.; Wang, J.; Viswanadhan, V. N.; Groneberg, R. D.; Zhan, J.; Suzuki, H.; Toro, A.; Mareska, D. A.; Clarke, D. E.; Harvey, D. M.; Burgess, L. E.; Laird, E. R.; Askew, B.; Ng, G. Identification of a Nonpeptidic and Conformationally Restricted Bradykinin 1 Antagonist with Anti-Inflammatory Activity. *J. Med. Chem.* **2007**, *50*, 607−610.

(13) Compound **2** and all analogues reported herein possess two benzylic amines: one present as an amide and the other as an amine. For purposes of clarification, the amine is marked with an asterisk in Figure 1 and is referred to as the "benzylic amine" throughout the text.

(14) Ni, A.; Chai, K. X.; Chao, L.; Chao, J. Molecular Cloning and Expression of Rat Bradykinin B1 Receptor. *Biochim. Biophys. Acta* **1998**, *1442*, 177−185.

(15) Macneil, T.; Bierilo, K. K.; Menke, J. G.; Hess, J. F. Cloning and Pharmacological Characterization of a Rabbit Bradykinin B1 Receptor. *Biochim. Biophys. Acta* **1995**, *1264*, 223−228.

(16) Corey, E. J.; Bakshi, R. K.; Shibata, S. Highly Enantioselective Borane Reduction of Ketones Catalyzed by Chiral Oxazaborolidines. Mechanism and Synthetic Implications. *J. Am. Chem. Soc.* **1987**, *109*, 5551−5553.

(17) There were other potent piperidine replacements identified as part of the SAR study in Table 1 (e.g., the 1-morpholino analog **17**), but those compounds suffered from poor pharmacokinetic properties (data not shown) and are not discussed further in this study.

(18) Regoli, D. C.; Marceau, F.; Lavigne, J. Induction of B1-Receptors for Kinins in the Rabbit by a Bacterial Lipopolysaccharide. *Eur. J. Pharmacol*. **1981**, *71*, 105−115.

(19) McLean, P. G.; Perretti, M.; Ahluwalia, A. Kinin B(1) Receptors and the Cardiovascular System: Regulation of Expression and Function. *Cardiovasc. Res.* **2000**, *48*, 194−210.

(20) Dong, H.; Sun, H.; Magal, E.; Ding, X.; Kumar, G. N.; Chen, J.; Johnson, E.; Manning, B. H. Inflammatory Pain in the Rabbit: A New, Efficient Method for Measuring Mechanical Hyperalgesia in the Hind Paw. *J. Neurosci. Methods* **2007**, submitted for publication.

JM070055C