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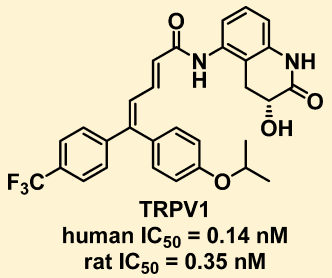
Discovery of Novel 5,5-Diarylpentadienamides as Orally Available Transient Receptor Potential Vanilloid 1 (TRPV1) Antagonists

Osamu Saku,* Hiroshi Ishida, Eri Atsumi, Yoshiyuki Sugimoto, Hiroshi Kodaira, Yoshimitsu Kato, Shiro Shirakura, and Yoshisuke Nakasato

Fuji Research Park, Kyowa Hakko Kirin Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Suntou-gun, Shizuoka 411-8731, Japan

Supporting Information

ABSTRACT: We have developed a novel and potent chemical series of 5,5-diphenylpentadienamides for targeting TRPV1 in vitro and in vivo. In this investigation, we examined a variety of replacements for the 5-position of dienamides with the goal of addressing issues related to pharmacokinetics. Our data suggest that substitution with alkoxy groups on the phenyl ring at the 5-position increases their ability to penetrate the blood−brain barrier. This investigation culminated in the discovery of compound (*R*)-**36b**, which showed a good pharmacokinetic profile. In vivo, compound (*R*)-**36b** was found to be effective at reversing mechanical allodynia in rats in a dose-dependent manner, and it reversed thermal hyperalgesia in a model of neuropathic pain induced by sciatic nerve injury.



INTRODUCTION

During more than 10 years since the cloning of transient receptor potential cation channel, subfamily V, member 1 (TRPV1), by Julius and colleagues in 1997, compelling data have been accumulating on the role of this channel.[1] These breakthrough studies led to our understanding of the molecular mechanisms involved in the responses of sensory neurons to noxious thermal and chemical stimuli. TRPV1 is a nonselective cation channel primarily expressed on unmyelinated pain-sensing nerve fibers (C-fibers) and small Aδ fibers in the dorsal root and trigeminal ganglia.[2] The activation of TRPV1 by a diverse range of stimuli including protons, heat, noxious chemicals such as resiniferatoxin[3] and capsaicin,[4] which is the pungent component of hot chili peppers, and endogenous substances such as anandamide[5] and lipoxygenase products[6] leads to an influx of calcium and sodium ions through the channel. An increase in intracellular calcium ions results in the excitation of primary sensory neurons and ultimately in the central perception of pain.[7] These diverse stimuli not only directly activate TRPV1 but also sensitize and reduce the activation thresholds of the channel to other stimuli, suggesting that blocking the activation of this receptor by desensitization or antagonism would have considerable therapeutic utility.[8,9] The clinical uses of TRPV1 agonists, however, are limited because of their gross neurotoxic effects.[10] In contrast, selective and potent TRPV1 antagonists structurally unrelated to the exogenous agonist, capsaicin, have been described in the literature and offered a rapid onset of analgesic action with potentially fewer side effects.[11]

To better understand the pharmacology of TRPV1, we sought to identify novel potent TRPV1 antagonists that would provide the ability to perform a broad assessment of the potential of this receptor for pain management. The search for our own TRPV1 antagonists started with compounds **1** and **2** identified by high-throughput screening of our own chemical library of TRPV1 agonists (Figure 1). These enamide and dienamide exhibited

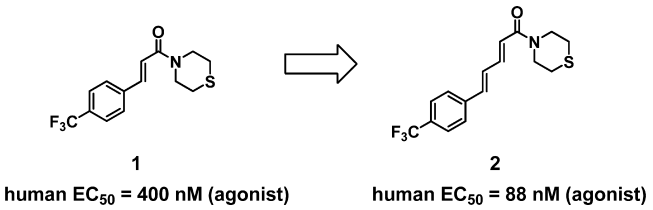


Figure 1. Enamide (**1**) and dienamide (**2**) identified as TRPV1 agonists through high-throughput screening.

agonistic activities in the capsaicin-induced Ca^{2+} influx assay in human TRPV1-expressing 293 Epstein−Barr virus nuclear antigen (EBNA) cells with EC_{50} values of 400 and 88 nM, respectively.

Small molecule antagonists of TRPV1 have recently been discovered as potential therapeutics for pain.[12] Some *N*-arylcinnamides were reported to exhibit potent TRPV1 antagonistic effects and moderate oral bioavailability in rats.[12a] Our results suggest that the agonists with a dienamide moiety afford superior binding affinity compared to the agonists with the enamide moiety, which is a prominent structure among the extensively characterized TRPV1 antagonists.[11b,13] Although it was a concern that the dienamide structure would be unstable, piperine (1-piperoylpiperidine), a 5-aryldienamide alkaloid from black pepper and hot peppers, was found to be stable in methanol for several weeks and under different temperature storage conditions.[14] The superiority of the dienamide structure was consistent with previous studies suggesting that the lipophilic side chain interacts with a hypothetic hydrophobic binding site on TRPV1.[15] Moreover, it was apparent that an alternative strategy for increasing the intrinsic lipophilicity of the compounds was desirable in order to design more potent compounds because a compound that would cross the

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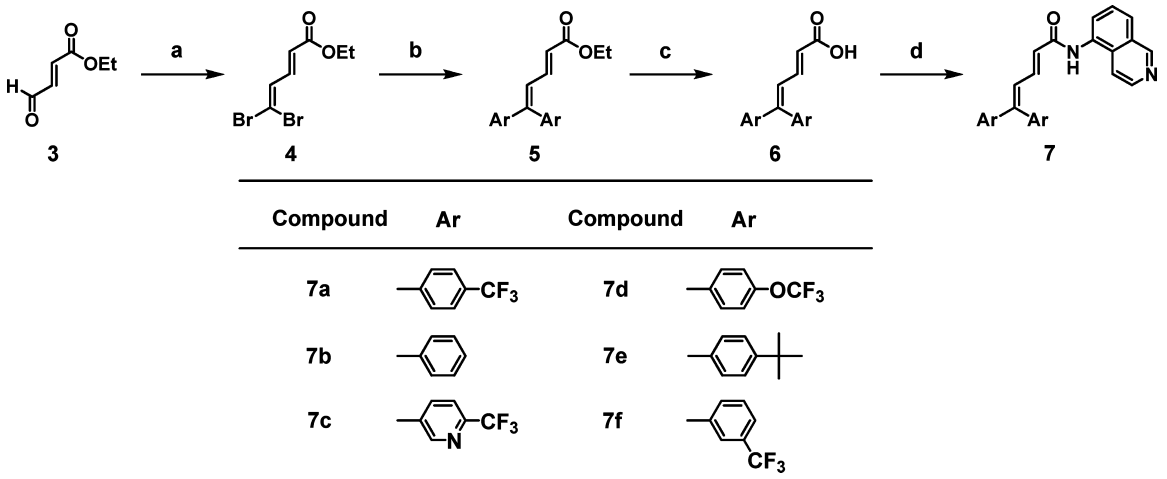
Scheme 1. Synthesis of Pentadienamides Possessing Two Identical Aryl Groups at the 5-Position[a]



[a]Reagents and conditions: (a) CBr_4, PPh_3, CH_2Cl_2; (b) $ArB(OH)_2$, $Pd(PPh_3)_4$, aq Na_2CO_3, dioxane; (c) $LiOH{\cdot}H_2O$, MeOH, H_2O, THF; (d) 5-aminoisoquinoline, WSC·HCl, $HOBt{\cdot}H_2O$, DMF.

blood–brain barrier was preferable for achieving good efficacy in vivo.[16] Therefore, we developed a strategy to enhance the potency and optimize the pharmacokinetic properties in this series of compounds by systematically exploring the compounds with a dienamide structure.

It has previously been reported that subtle structural modifications to TRPV1 modulators can result in a reversal of activity.[17] We have successfully designed and synthesized potent and selective antagonists of the human TRPV1, based on information derived from small molecule agonists. Herein, we report the in vitro structure–activity relationships and in vivo characterization of a series of 5,5-diarylpentadienamide analogues as potent and selective TRPV1 antagonists, which culminated in the identification of our first TRPV1 clinical candidate, (2*E*,4*Z*)-*N*-[(3*R*)-3-hydroxy-2-oxo-1,2,3,4-tetrahydro-5-quinolyl]-5-(4-isopropoxyphenyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide ((*R*)-**36b**).

CHEMISTRY

The synthesis of pentadienamide analogues possessing two identical aryl groups at the 5-position was easily accomplished, as shown in Scheme 1. Ethyl 5,5-dibromopentadienoate (**4**) was obtained in a good yield from the reaction of aldehydes **3** with triphenylphosphine and carbon tetrabromide. A standard Suzuki coupling reaction was first investigated, utilizing tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$] as the catalyst source and sodium carbonate as the base in dioxane. The expected ethyl diarylpentadienoates **5** were formed in moderate to good yields, followed by hydrolysis of the resultant ethyl esters to give the desired carboxylic acids **6**. The final steps to prepare amides **7** required the coupling of acids **6** with 5-aminoisoquinoline.

The approach for the synthesis of pentadienamide analogues with two different aryl groups at the 5-position is outlined in Scheme 2. It is known that the rates of palladium catalyzed cross-coupling reactions of (*E*)- and (*Z*)-1-bromo-1-alkenes are substantially different. Shen reported that the soft ligand tris(2-furyl)phosphine (TFP) is very effective in the palladium-catalyzed stereoselective synthesis of (*Z*)-1-aryl(alkenyl)-1-bromo-1-alkenes from 1,1-dibromo-1-alkenes.[18] However, the Suzuki coupling reaction of ethyl 5,5-dibromopentadienoate (**4**) with 4-trifluoromethylphenylboronic acid using the same coupling condition gave a mixture of ethyl 5-bromo-5-(4-trifluoromethylphenyl)-2,4-pentadienoate with a 2:1 ratio of the (2*E*,4*Z*)- and (2*E*,4*E*)-isomers in a yield of 64%, with concomitant formation of ethyl (*E*)-5,5-bis(4-trifluoromethylphenyl)-2,4-pentadienoate with a yield of 4%. The structures of the two stereoisomers were confirmed by the nuclear Overhauser effect (NOE) enhancement observed between the hydrogen at the 3-position and the hydrogen on the benzene ring. Because it was difficult to isolate the desired (2*E*,4*Z*)-isomer **8a** from the double substituted compound, we screened several conditions to improve the yield and selectivity. The reaction proceeded faster when $Pd(PPh_3)_4$ was used instead of $Pd_2(dba)_3$ and TFP. Among the solvents examined, tetrahydrofuran (THF) was found to best reduce the formation of the double substituted compound while retaining the yield of **8a**. The optimized reaction conditions afforded compound **8a** in 66% isolated yield, with the formation of the isomer in 20% yield. For the second coupling reaction, the combination of $Pd_2(dba)_3$ and the TFP ligand gave the best result. The esters **9** were transformed into amide derivatives using the same method described for the synthesis of compound **7**. Boronic acid pinacol esters, obtained from the bromide, successfully gave ethyl 5-aryl-5-bromo-2,4-pentadienoate **8**.

An alternative route was developed in order to improve the regioselectivity and to further confirm the structure (Scheme 3). Treatment of phenylacetylene (**12**) with *n*-BuLi and reaction with ethyl chloroformate gave the acetylenic ester **13**. The 3-iodide in compound **14**, prepared by the addition of NaI to compound **13**,[19] was displaced by a 4-trifluoromethylphenyl group under the conditions described above. Ester **15** was converted to aldehyde **17** by reducing the ester to an alcohol, then oxidizing it. A Horner–Emmons reaction at the aldehyde for carbon chain elongation gave compound **18**. The ester **18** was transformed into amide derivatives **20** via the same method described in Scheme 2.

The synthetic route employed to prepare 5-alkylsubstituted or 5-monosubstitued analogues of pentadienamides is outlined in Scheme 4. The Horner–Emmons reaction with ketone **21a**,**b** or aldehyde **21c** afforded a mixture of (*E*)- and (*Z*)-acrylate esters, followed by purification using silica gel column chromatography to give (*E*)-acrylate esters **22**. In a similar manner, 5-alkyl-substituted

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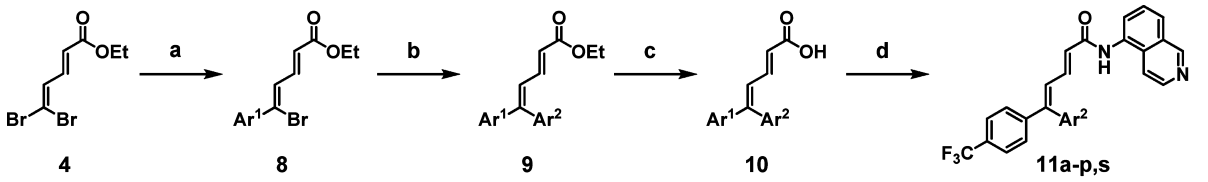
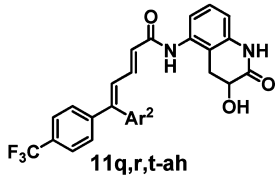
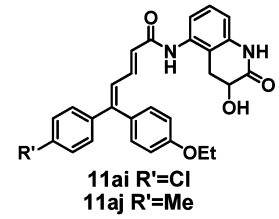
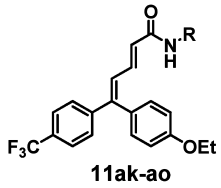
Scheme 2. Synthesis of Pentadienamides Possessing Two Different Aryl Groups at the 5-Position[a]



Compound	Ar^2	Compound	Ar^2	Compound	Ar^2
11a		11g		11m	
11b	OMe	11h		11n	
11c	F	11i		11o	
11d	OH	11j		11p	
11e	CN	11k	Me	11s	NMe_2
11f		11l			







Compound	Ar^2	Compound	Ar^2	Compound	Ar^2
11q	F	11x		11ad	OCF_3
11r		11y		11ae	OEt
11t	NMe_2	11z	NMe_2	11af	EtO
11u		11aa		11ag	OEt
11v		11ab	OH	11ah	OEt
11w	NMe_2	11ac	OMe		

Compound	R	Compound	R	Compound	R
11ak		11am		11ao	
11al		11an			

[a]Reagents and conditions: (a) $Ar^1B(OH)_2$, $Pd(PPh_3)_4$, aq Na_2CO_3, THF; (b) $Ar^2B(OH)_2$, $Pd_2(dba)_3$, TFP, aq Na_2CO_3, dioxane or $Ar^2Sn(n\text{-}Bu)_3$, toluene; (c) LiOH·H_2O, MeOH, H_2O, THF; (d) RNH_2, WSC·HCl, HOBt·H_2O, DMF.

or 5-monosubstituted pentadienamides **27** were prepared from esters **22**.

The 5-(4-morpholinomethylphenyl) analogue **32** was prepared from the 5-(4-formylphenyl) analogue **31**, which was obtained by hydrolysis of enamine **30**, utilizing the reductive amination of aldehyde with sodium triacetoxyborohydride (Scheme 5). For the preparation of the 5-(4-alkoxyphenyl) analogues **36** in Scheme 6, ethyl (2*E*,4*Z*)-5-(4-hydroxyphenyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (**33**) was subjected to the Mitsunobu reaction to introduce the desired alkoxy substituent, while in the case of **36g**, chloroacetonitrile with K_2CO_3 was employed (Scheme 7). Direct Mitsunobu

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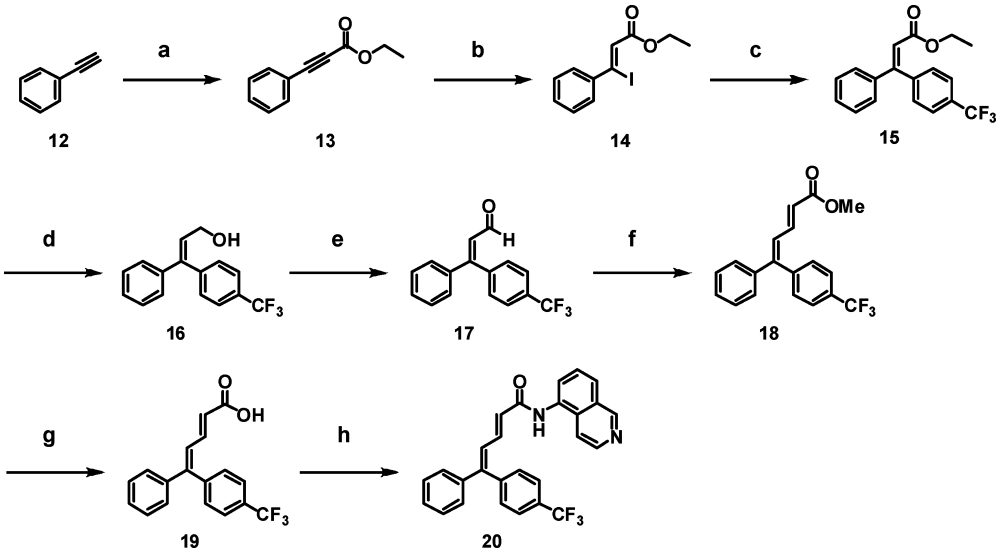
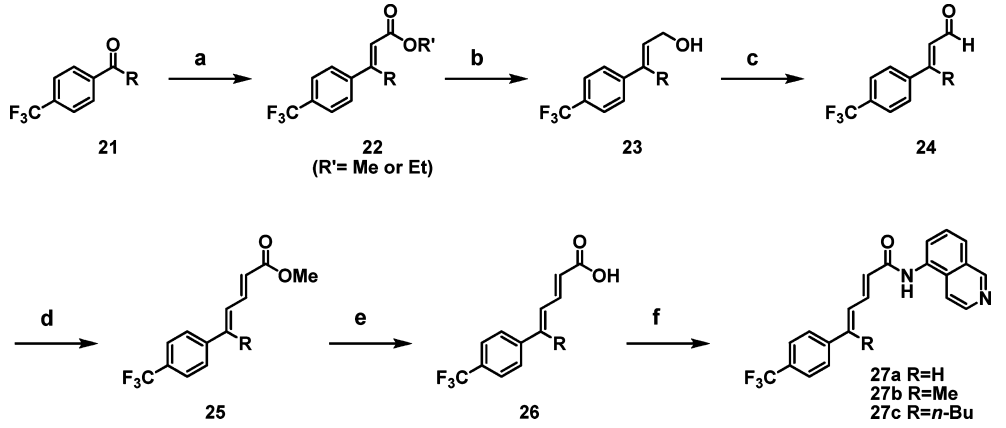
Scheme 3. Alternative Method for the Synthesis of Pentadienamides Possessing Two Different Aryl Groups at the 5-Position[a]



[a]Reagents and conditions: (a) ethyl chloroformate, *n*-BuLi, THF; (b) NaI, AcOH; (c) 4-(trifluoromethyl)phenylboronic acid, $Pd_2(dba)_3$, TFP, aq Na_2CO_3, dioxane; (d) $(i\text{-}Bu)_2AlH$, THF; (e) MnO_2, CH_2Cl_2: (f) $Ph_3P{=}CHCOOMe$, CH_2Cl_2; (g) $LiOH{\cdot}H_2O$, MeOH, H_2O, THF; (h) 5-aminoisoquinoline, WSC·HCl, $HOBt{\cdot}H_2O$, DMF.

Scheme 4. Synthesis of Pentadienamides Possessing Alkyl Group at the 5-Position[a]



[a]Reagents and conditions: (a) $Ph_3P{=}CHCOOMe$, CH_2Cl_2 or $(EtO)_2POCH_2COOEt$, NaH, DME; (b) $(i\text{-}Bu)_2AlH$, THF; (c) MnO_2, CH_2Cl_2; (d) $Ph_3P{=}CHCOOMe$, CH_2Cl_2; (e) $LiOH{\cdot}H_2O$, MeOH, H_2O, THF; (f) 5-aminoisoquinoline, WSC·HCl, $HOBt{\cdot}H_2O$, DMF.

coupling of oxetane-3-ol to the phenol analogue **33** was unsuccessful, so a newly developed synthetic route was used to prepare compound **45** which has an oxetane ring (Scheme 8). Coupling of 4-bromophenol (**37**) with diacetin provided the ether **38**, which was hydrolyzed to **39** with lithium hydroxide. The tosylation of a single hydroxyl group of the dialcohol, followed by sodium hydride-mediated cyclization to an oxetane ring, provided bromide **41**. The ethyl pentadienoate **43** was prepared from the coupling of bromide **41** with boronate **42**, which was synthesized by the coupling of bromide **8a** with bis(pinacolato)diboron. The desired pentadinamide **45** was synthesized from ester **43** as described above.

Scheme 9 describes the synthesis of pentadienamide derivatives with a 2-substituted pyridine at the 5-position. A nucleophilic substitution of the *o*-chlorine on the pyridine ring by amines gave the corresponding products **47**, followed by Weinreb amides **48** formation. The reactions of the Weinreb amides **48** with Grignard reagent led to the expected ketone **49**. The corresponding ethyl 3,3-diarylacrylates **50** were prepared by a Horner−Emmons reaction, followed by conversion to dinenoic acid esters **53** by the same strategy shown in Scheme 4. Hydrolysis of **53** with lithium hydroxide, followed by water-soluble carbodiimide (WSC) mediated coupling to the amine, provided the pentadienamide **55**.

The requisite heteroaromatic amines were either commercially available or readily prepared following published methods.[20] Optically active 5-amino-3-hydroxy-2-oxo-1,2,3,4-tetrahydroquinoline was produced through a diastereomer salt resolution using optically active tartaric acid as the resolving reagent. Resolution of the racemic amine with L- and D-tartaric acids gave chiral amines (3*R*)-5-amino-3-hydroxy-2-oxo-1,2,3,4-tetrahydroquinoline (99.0% ee) and (3*S*)-5-amino-3-hydroxy-2-oxo-1,2,3,4-tetrahydroquinoline (99.9% ee) in good yield, respectively. Their absolute configurations were determined by an X-ray crystallographic analysis.

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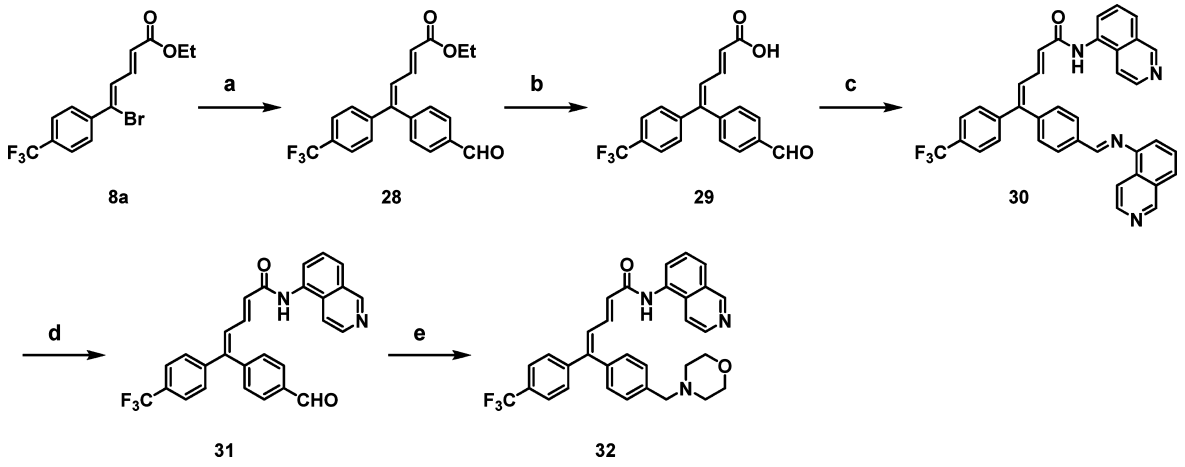
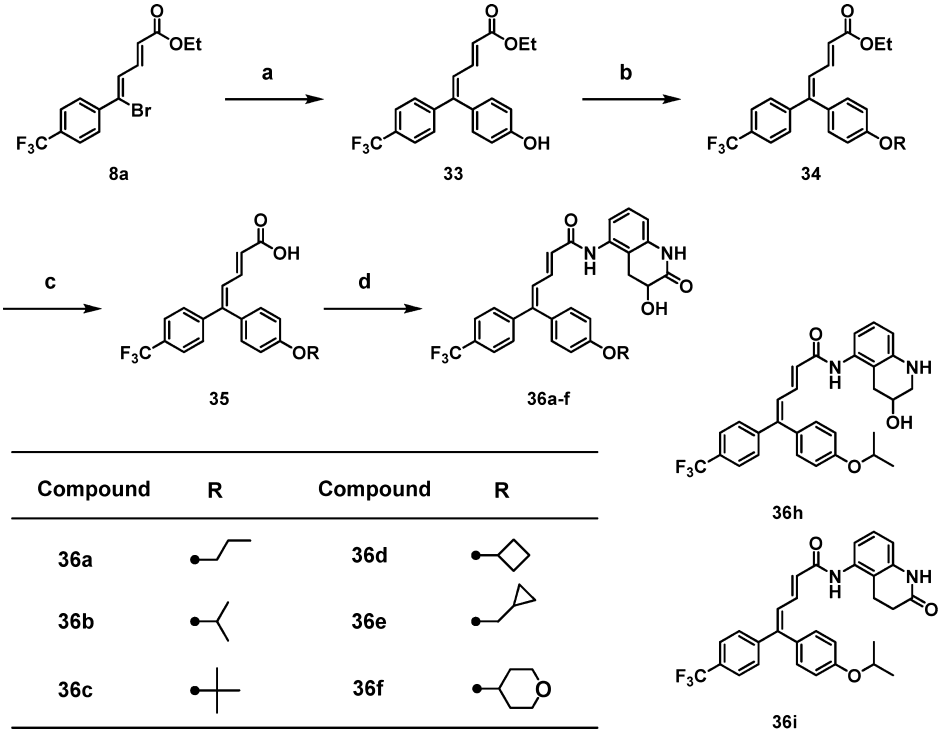
Scheme 5. Synthesis of Pentadienamides Possessing 4-(Morpholinomethyl)phenyl Group at the 5-Position[a]



[a]Reagents and conditions: (a) 4-formylphenylboronic acid, $Pd_2(dba)_3$, TFP, aq Na_2CO_3, dioxane; (b) $LiOH·H_2O$, MeOH, H_2O, THF; (c) 5-aminoisoquinoline, WSC·HCl, $HOBt·H_2O$, DMF; (d) 1 M HCl, THF; (e) morpholine, $NaBH(OAc)_3$, CH_2Cl_2.

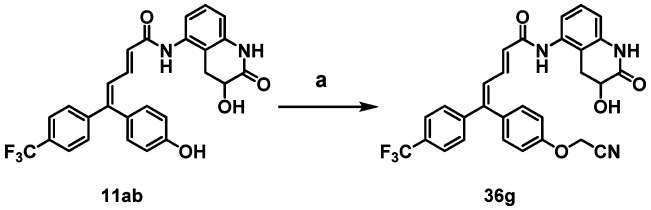
Scheme 6. Synthesis of Pentadienamides Possessing 4-Alkoxyphenyl Group at the 5-Position[a]



Compound	R	Compound	R
36a		36d	
36b		36e	
36c		36f	

[a]Reagents and conditions: (a) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol, $Pd_2(dba)_3$, TFP, aq Na_2CO_3, dioxane; (b) ROH, DEAD, PPh_3, toluene; (c) $LiOH·H_2O$, MeOH, H_2O, THF; (d) $R'NH_2$, WSC·HCl, $HOBt·H_2O$, DMF.

Scheme 7. Synthesis of Pentadienamides Possessing 4-(Cyanomethoxy)phenyl Group at the 5-Position[a]



[a]Reagents and conditions: (a) chloroacetonitrile, K_2CO_3, DMF.

RESULTS AND DISCUSSION

The functional antagonist activity of compounds prepared in this study at the TRPV1 receptor was determined with a Ca^{2+} influx assay by measuring the effect on capsaicin evoked increase in intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) using human or rat TRPV1-expressing 293 EBNA cells. $[Ca^{2+}]_i$ response was monitored using a fluorescent probe in conjunction with a fluorometric imaging plate reader (FLIPR). Their functional activity is reported as the IC_{50}.

The search for a new TRPV1 antagonist that is capable of crossing the blood–brain barrier in order to achieve efficacy in

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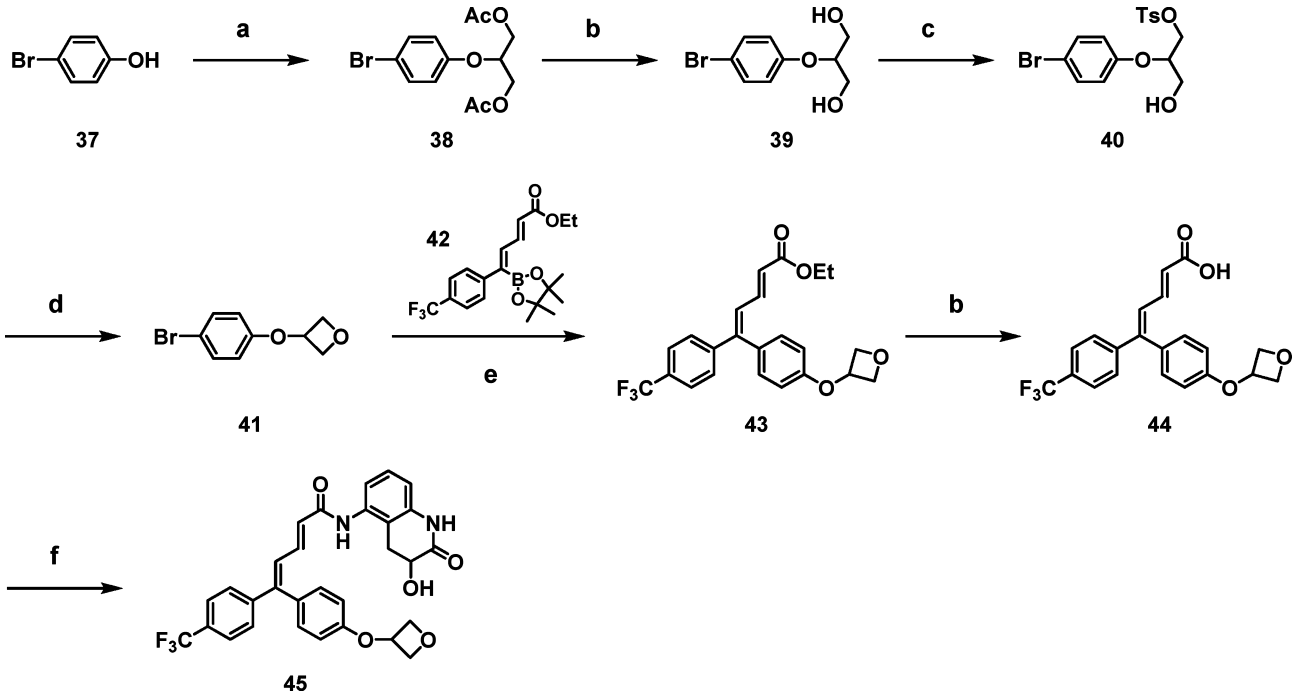
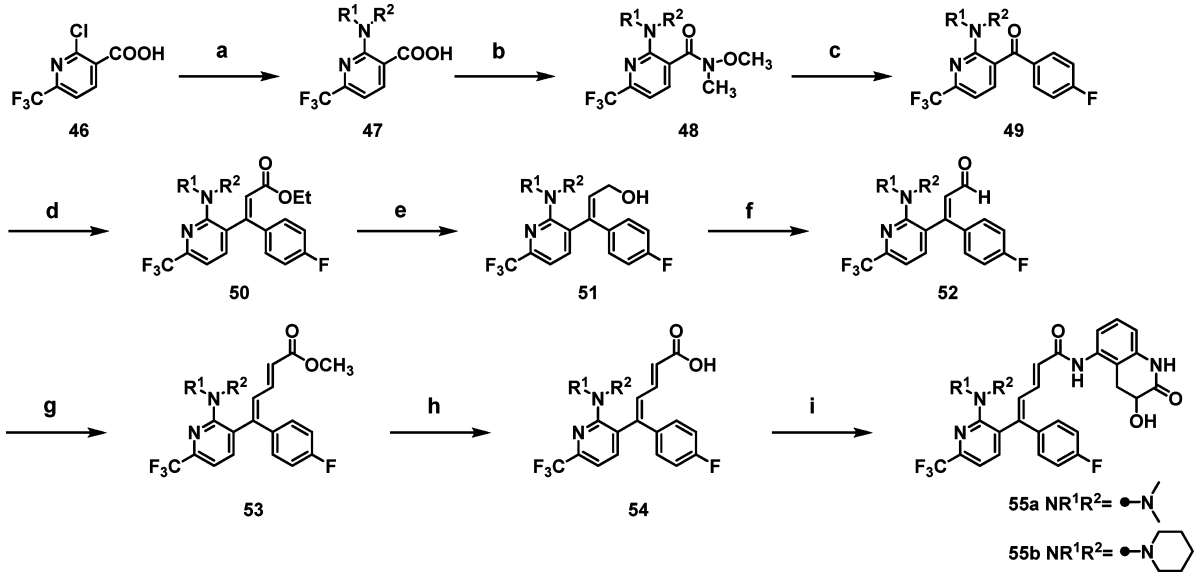
Scheme 8. Synthesis of Pentadienamides Possessing 4-(Oxetan-3-yloxy)phenyl Group at the 5-Position[a]



[a]Reagents and conditions: (a) diacetin, DEAD, PPh_3, toluene; (b) $LiOH·H_2O$, MeOH, H_2O, THF; (c) TsCl, NaH, THF; (d) NaH, THF; (e) $Pd(PPh_3)_4$, aq Na_2CO_3, THF, (f) 5-amino-3-hydroxy-3,4-dihydroquinolin-2(1*H*)-one, WSC·HCl, $HOBt·H_2O$, DMF.

Scheme 9. Synthesis of Pentadienamides Possessing 2-Substituted Pyridine at the 5-Position[a]



[a]Reagents and conditions: (a) R^1R^2NH, H_2O; (b) MeNHOMe·HCl, WSC·HCl, $HOBt·H_2O$, Et_3N, DMF; (c) (4-fluorophenyl)magnesium bromide, THF; (d) $(EtO)_2POCH_2COOEt$, NaH, THF; (e) $(i\text{-}Bu)_2AlH$, THF; (f) MnO_2, CH_2Cl_2; (g) $Ph_3P{=}CHCOOMe$, CH_2Cl_2; (h) $LiOH·H_2O$, MeOH, H_2O, THF; (i) 5-amino-3-hydroxy-3,4-dihydroquinolin-2(1*H*)-one, WSC·HCl, $HOBt·H_2O$, DMF.

a rodent model of pain started with pentadienamide derivatives **1** and **2**, identified by high-throughput screening of our compound library as TRPV1 agonists (Figure 1). The incorporation of another aryl group at the 5-position led to increased agonist potency (data not shown). Additionally, these compounds were successfully converted into antagonists by modifying the thiomorpholino group in the amide moiety, based on a previous report in which the intrinsic efficacy at TRPV1 was shown to be especially sensitive to structural changes in the amide region.[17] These findings indicated that the 5,5-diaryl-pentadienamide is a suitable lead structure for the development of novel TRPV1 antagonists. On the basis of these leads, we started a research effort to improve the antagonistic activity, as well as increase metabolic stability, of these compounds.

The pentadienamide analogues possessing two identical aryl groups at the 5-position were tested for their ability to block the capsaicin-mediated activation of human and rat TRPV1 channels, and their pharmacokinetic profiles were assessed in rats.

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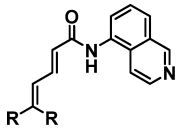
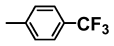
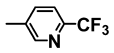
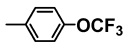
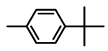
The results are shown in Table 1. The removal of trifluoromethyl groups at the benzene ring of **7a** resulted in an approximately

Table 1. In Vitro TRPV1 Activities and Intrinsic Clearance in Rat Liver Microsomes for Pentadienamide Analogues Possessing Two Identical Aryl Groups at the 5-Position



Compound	R	human IC_{50}[a] (nM)	rat IC_{50}[a] (nM)	rat CLint[b] (L/h/kg)
7a		0.42	2.0	1200
7b		37	74	410
7c		130	500	57
7d		6.6	12	740
7e		0.18	0.31	10000
7f		1.6	2.7	1300

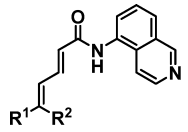
[a]IC_{50} values based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human or rat TRPV1-expressing 293 EBNA cells. Each IC_{50} value reported represents an average of at least three independent experiments with four replicates at each concentration. [b]Intrinsic clearance calculated from the disappearance rate of compound in rat liver microsomes. $n = 2$.

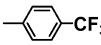
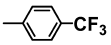
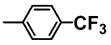
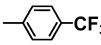
100-fold decrease in potency on the human TRPV1 (compound **7b**). The pyridyl isomer **7c** was much less potent in comparison with **7a**, while the pharmacokinetic profile was improved because of the reduced hydrophobicity. Replacement of the trifluoromethyl group with a trifluoromethoxy group also led to reduced potency (compound **7d**), while the *tert*-butyl analogue **7e** was more potent than **7a**. Moving the trifluoromethyl group to the meta position decreased the potency, as evidenced in compound **7f**. Compound **7e** was the most potent but was not further considered because of its high rate of clearance.

To achieve an appropriate balance between potency and pharmacokinetic properties, we sought to optimize the pentadienamide analogues with two different aryl groups at the 5-position. The first replacement examined the effect of two aryl groups at the 5-position, as shown in Table 2. A loss in activity was prominent for compound **20**, which was lacking the trifluoromethylphenyl group from the left benzene ring of compound **7a**. In contrast, a 3-fold increase in potency was observed for the isomeric compound **11a**. This promising result prompted us to investigate the effects of substitution on the right phenyl ring.

For these subsequent studies, a small library was designed to probe substitutions of the right phenyl ring, as exemplified in Table 3. The introduction of a methoxy group at the para position (**11b**) had minimal impact. However, substitution of this ring with a fluoro group (**11c**) improved the potency compared to **11a**. A hydrophilic group, such as a hydroxyl (**11d**) or cyano (**11e**) group, at this position decreased the potency, which suggests that the substitution at this position is slightly sensitive to the polar effect of substituents. Conversely, introduction of a morpholino group at the para-position led to the retention of an

Table 2. In Vitro TRPV1 Activities for Pentadienamide Analogues Possessing Two Different Aryl Groups at the 5-Position



Compound	R^1	R^2	human IC_{50}[a] (nM)
7a			0.42
20			4.1
11a			0.14
7b			37

[a]IC_{50} values based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human TRPV1-expressing 293 EBNA cells. Each IC_{50} value reported represents an average of at least three independent experiments with four replicates at each concentration.

acceptable potency, while the methylene linker did not improve the activity in comparison to **11f**.

To improve the potency, we next investigated the effects of introducing heterocyclic groups into the molecule. Replacement of the right phenyl ring with an unsubstituted 2-thienyl group (**11g**) provided a slight decrease in the in vitro activity. The position of attachment was also investigated, as shown by the comparison of the 2-thienyl (**11g**) and 3-thienyl (**11h**) analogues. Compound **11h** was about 2-fold more potent than compound **11g**. The 2-furyl (**11i**) or 3-furyl (**11j**) analogues had a lower affinity compared to compound **11g**. The addition of substituents on the heterocyclic ring had a significant effect on the potency, as shown by the difference between **11i** and **11k**. The 3-methylfuran-2-yl analogue **11k** had about a 10-fold higher affinity than the unsubstituted 2-furyl analogue **11i** at the human TRPV1 receptor. The 3-pyridyl (**11l**), 4-pyridyl (**11m**), and 5-pyrimidinyl (**11n**) analogues were also synthesized because these modifications were expected to reduce the lipophilicity of the compound. However, these compounds exhibited poor activity. For alkyl substituents, the bulky and hydrophobic substituted analogues **27c** (*n*-butyl) and **11o** (cyclohexenyl) gave higher antagonistic activities than the relatively polar group analogue **11p**, while nonsubstituted analogue (**27a**), as well as the methyl substituted analogue (**27b**), was not tolerated.

Compounds containing the 3-hydroxy-3,4-dihydroquinolin-2(1*H*)-one rather than the isoquinoline heterocycle were also synthesized and evaluated. As shown in Table 4, in the 3-hydroxy-3,4-dihydroquinolin-2(1*H*)-one series, a slight decrease in the in vitro potency was observed while the metabolic stability was improved compared to the isoquinoline analogues (e.g., compare **11c**, **11i**, **11s** to **11q**, **11r**, **11t**, respectively). Because we assumed that decreased lipophilicity would improve the pharmacokinetic profile, 2-amino-substituted pyridine and pyrimidine analogues were designed and synthesized.

As can be seen from compounds **11t–aa** in Table 5, we again found that pyridyl (**11w–y**) or pyrimidyl (**11z,aa**) analogues were detrimental to the potency while even a small hydrophobic modification at this position with nitrogen-containing heterocycles, such as pyrrolidine and piperazine, resulted in increased potency. This indicated that the addition of hydrophobic groups is favorable. The in

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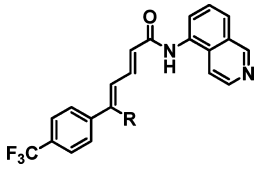
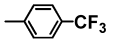
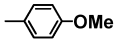
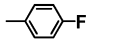
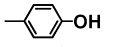
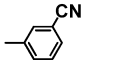
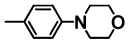
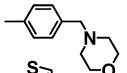
Table 3. In Vitro TRPV1 Activities for Pentadienamide Analogues Possessing Two Different Aryl Groups at the 5-Position



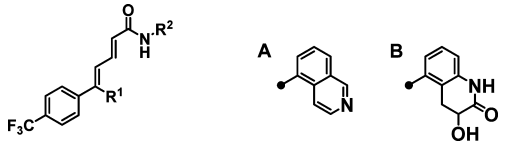
Compound	R	human IC_{50}[a] (nM)	rat IC_{50}[a] (nM)
7a		0.42	2.0
11a		0.14	0.90
11b		0.15	0.62
11c		0.072	0.30
11d		8.2	20
11e		2.1	7.0
11f		0.58	1.2
32		1.6	4.2
11g		0.96	2.6
11h		0.49	1.7
11i		3.3	7.4
11j		3.3	9.9
11k		0.34	2.3
11l		34	110
11m		16	84
11n		240	720
27a	−H	940	1600
27b	−Me	950	330
27c	−*n*-Bu	6.0	6.4
11o		0.33	1.1
11p		26	70

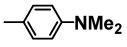
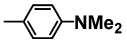
[a] IC_{50} values based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human or rat TRPV1-expressing 293 EBNA cells. Each IC_{50} value reported represents an average of at least three independent experiments with four replicates at each concentration.

vitro inhibitory activities of the compounds revealed a significant relationship between their ring size and potency.

Next, we turned our attention to the alkoxy substituents, which were shown to be capable of replacing the trifluoromethyl group in Table 6. We therefore introduced a variety of alkoxy groups, with the trifluoromethyl substituent fixed on the left-side phenyl ring. Again, the activity was apparently dependent on the size of

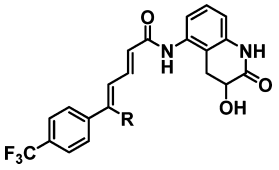
Table 4. In Vitro TRPV1 Activities and Intrinsic Clearance in Human Liver Microsomes for Pentadienamide Analogues with Different Amide Moieties

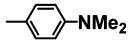
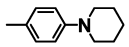
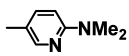
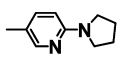
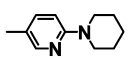
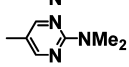
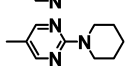


Compound	R^1	R^2	human IC_{50}[a] (nM)	rat IC_{50}[a] (nM)	human[b] CLint (L/h/kg)
11c		A	0.07	0.30	N.D.[c]
11q		B	0.11	0.78	30
11i		A	3.3	7.4	92
11r		B	6.4	15	22
11s		A	0.36	0.63	620
11t		B	1.4	3.1	230

[a] IC_{50} values based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human or rat TRPV1-expressing 293 EBNA cells. Each IC_{50} value reported represents an average of at least three independent experiments with four replicates at each concentration. [b] Intrinsic clearance calculated from the disappearance rate of compound in human liver microsomes. n = 2. [c] N.D. = not determined.

Table 5. In Vitro TRPV1 Activities for Pentadienamide Analogues with Tertiary Amine Substituted Analogues



Compound	R	human IC_{50}[a] (nM)	rat IC_{50}[a] (nM)
11u		0.38	1.1
11t		1.4	3.1
11v		0.26	0.97
11w		6.5	9.6
11x		2.2	7.3
11y		0.65	0.92
11z		26	28
11aa		0.43	1.8

[a] IC_{50} values based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human or rat TRPV1-expressing 293 EBNA cells. Each IC_{50} value reported represents an average of at least three independent experiments with four replicates at each concentration.

the hydrophobic alkoxy group. The addition of the oxetane (**45**) and tetrahydro-2*H*-pyran (**36f**) rings were also beneficial, while the cyanomethoxy group (**36g**) led to a slight loss of activity.

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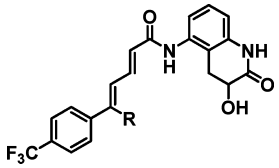
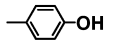
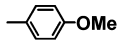
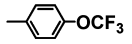
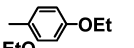
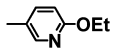
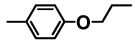
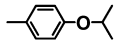
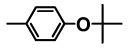
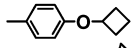
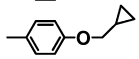
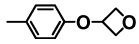
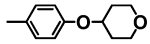
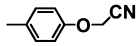
Table 6. In Vitro TRPV1 Activities for Pentadienamide Analogues with Alkoxy Substituted Analogues



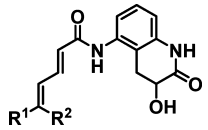
Compound	R	human IC_{50}[a] (nM)	rat IC_{50}[a] (nM)
11ab		33	160
11ac		0.25	0.75
11ad		0.14	0.32
11ae		0.39	0.67
11af		1.6	2.9
11ag		0.90	1.6
11ah		3.9	9.1
36a		0.080	0.23
36b		0.14	0.35
36c		0.091	0.33
36d		0.041	0.29
36e		0.13	0.36
45		1.1	2.6
36f		0.58	1.1
36g		1.4	2.8

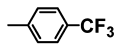
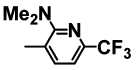
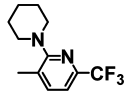
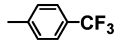
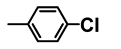
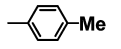
[a]IC_{50} values based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human or rat TRPV1-expressing 293 EBNA cells. Each IC_{50} value reported represents an average of at least three independent experiments with four replicates at each concentration.

We also examined the effect of the substituents on the left phenyl ring using other replacements. The inhibitory activity for the analogues in Table 7 was ranked in the following order: **11q** > **55b** > **55a**. Although the dimethylaminopyridine analogue **55a** and piperidylpiridine analogue **55b** were slightly less active than the trifluoromethylphenyl analogue **11q**, these results demonstrated that an N-substituted heterocyclic ring could serve as an isosteric replacement to the left phenyl ring. Chloro (**11ai**) or methyl (**11aj**) substituted analogues showed potencies comparable to that of the trifluoromethyl substituted analogue **11ae**.

With the 5-alkoxyphenylpentadienamide structure identified as a promising framework to obtain high-affinity TRPV1 ligands, we were interested in examining the effect of incorporating some of the quinolinone elements into the amide moiety. The results for studies of both the rat and the human receptors are shown in Table 8. The deletion of the carbonyl oxygen from compound **11ae** resulted in a slight increase in activity, while this is not the case for compound **36b**. To clarify the effects of

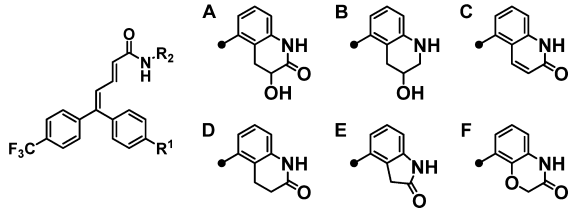
Table 7. In Vitro TRPV1 Activities for Pentadienamide Analogues: Variations to the Left Aryl Portion



Compound	R^1	R^2	human IC_{50}[a] (nM)	rat IC_{50}[a] (nM)
11q			0.11	0.78
55a			1.0	8.4
55b			0.61	2.1
11ae			0.39	0.67
11ai			0.24	0.84
11aj			0.40	1.6

[a]IC_{50} values based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human or rat TRPV1-expressing 293 EBNA cells. Each IC_{50} value reported represents an average of at least three independent experiments with four replicates at each concentration.

Table 8. In Vitro TRPV1 Activities for Pentadienamide Analogues: Variations to the Amide Portion



compound	R^1	R^2	human IC_{50}[a] (nM)	rat IC_{50}[a] (nM)
11ae	OEt	A	0.39	0.67
11ak	OEt	B	0.14	0.46
11al	OEt	C	0.22	0.90
11am	OEt	D	0.25	0.91
11an	OEt	E	1.3	9.7
11ao	OEt	F	15	57
36b	O-*i*-Pr	A	0.14	0.35
36h	O-*i*-Pr	B	0.16	0.55
36i	O-*i*-Pr	D	0.46	0.76

[a]IC_{50} values based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human or rat TRPV1-expressing 293 EBNA cells. Each IC_{50} value reported represents an average of at least three independent experiments with four replicates at each concentration.

the hydroxyl group on compound **11ae**, we prepared the quinolinone (**11al**) and 3,4-dihydroquinolin-2(1*H*)-one (**11am, 36i**) analogues. These modifications resulted in the retention of the activity. However, the indolin-2-one (**11an**) and 2*H*-benzo[*b*]-[1,4]oxazin-3(4*H*)-one (**11ao**) analogues suffered from a significant reduction of potency.

Because they exhibited excellent in vitro activities, the pharmacokinetic profiles of selected analogues were evaluated in male Sprague–Dawley rats (Table 9). Brain-to-plasma concentration ratio (K_p) is the most commonly used parameter to evaluate brain penetration and has been used as the primary parameter to

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Table 9. In Vitro and In Vivo Parameters of Selected TRPV1 Antagonists

compound	human IC_{50} [a] (nM)	rat IC_{50} [a] (nM)	C_{max} [b] (ng/mL)	$T_{1/2}$ [b] (h)	$AUC_{0-\infty}$ [b] (ng·h/mL)	Kp [c] brain	CYP3A4[d] % inhibition at 50 μmol/L
11ad	0.136 ± 0.014	0.320 ± 0.031	111	45	8250	0.29	21
11ae	0.389 ± 0.034	0.673 ± 0.135	141	23	5640	0.33	1.5
(*R*)-**11ae**	0.125 ± 0.048	0.302 ± 0.078	164	22	5900	N.D.[e]	9
(*S*)-**11ae**	0.141 ± 0.034	0.877 ± 0.134	97	16	2800	N.D.[e]	13
11ak	0.142 ± 0.008	0.459 ± 0.104	101	4.7	1090	0.91	19
11am	0.246 ± 0.024	0.910 ± 0.292	158	22	6010	0.61	2.6
36a	0.080 ± 0.020	0.231 ± 0.117	123	47	8110	0.34	16
36b	0.142 ± 0.033	0.353 ± 0.071	170	34	10400	0.18	12
(*R*)-**36b**	0.206 ± 0.041	0.322 ± 0.067	225	30	12000	0.20	10
(*S*)-**36b**	0.311 ± 0.083	0.769 ± 0.234	176	20	6230	N.D.[e]	13
36c	0.091 ± 0.028	0.325 ± 0.045	118	29	6020	0.23	7.9

[a] IC_{50} values based on inhibition of capsaicin (100 nM) induced influx of Ca^{2+} into human or rat TRPV1-expressing 293 EBNA cells. Each IC_{50} value reported represents an average of at least three independent experiments with four replicates at each concentration. [b] Pharmacokinetic parameters of TRPV1 antagonists after oral administration at a dose of 1 mg/kg to male rats. [c] Brain to plasma concentration ratio of TRPV1 antagonists at 7.5−8.3 h after oral administration at a dose of 10 or 30 mg/kg to male rats. [d] Inhibition percentages of TRPV1 antagonists for CYP3A4 activity in human liver microsomes. [e] N.D. = not determined.

Table 10. Pharmacokinetic Parameters for Compound (*R*)-36b Following iv and po Administration in Different Species

species	CL [a] (L h^{-1} kg^{-1}) (iv)	V_{dss} [a] (L/kg) (iv)	C_{max} [b] (ng/mL) (po)	$t_{1/2}$ [b] (h) (po)	*F* (%)
rat	0.0455 ± 0.0017	2.05 ± 0.18	225	29.8	54.5
dog	0.0301 ± 0.0026	1.61 ± 0.26	402 ± 82	37.2 ± 4.9	72.2 ± 6.7
monkey	0.0297 ± 0.0061	2.13 ± 0.83	312 ± 46	75.0 ± 3.9	95.0 ± 11.3

[a] 0.1 mg/kg in DMSO/PEG-400/saline = 5/50/45 (rat, dog) or in DMSO/Tween-80/saline = 0.4/1/98.6 (monkey), *n* = 3. [b] 1 mg/kg in 0.5 w/v% methylcellulose, *n* = 2 (rat) or 3 (dog, monkey).

optimize brain drug delivery in central nervous system (CNS) drug discovery.[21] Compounds were evaluated for their ability to penetrate the CNS by determining the K_p values in rats after oral administration. The K_p values of the 4-alkoxyphenyl analogues were substantially higher than those of the 4-aminophenyl analogues (data not shown), suggesting that the 4-alkoxyphenyl analogues have high potential as a treatment for neuropathic pain. The high lipophilicity of these compounds may account for the higher brain penetration. These derivatives showed similar pharmacokinetic profiles, exhibiting moderate oral bioavailabilities and CNS permeabilities, as well as a long half-life that may make them amenable to once-a-week administration. As shown in Table 9, negligible inhibitions of cytochrome P450 (CYP) indicated that there is likely to be an excellent safety profile for these compounds. Among the various compounds evaluated, compound **36b** showed a slight improvement in the C_{max} and AUC compared to those of other compounds. The solubility of **36b** in fed state simulated intestinal fluid at pH 6.4 was excellent (>500 μg/mL), and no inhibitory effect of this compound on human ether-a-go-go-related gene (hERG) encoded K channels was observed at 10 μM. The stereochemical effect was confirmed by a comparison of both enantiomers, having a hydroxyl group attached to the carbon atom of the quinolone ring linked to the amide nitrogen. The (*R*)-enantiomers (*R*)-**11ae** and (*R*)-**36b** were more potent than the (*S*)-enantiomers in both human and rat capsaicin-mediated assays. These results indicate that the *R*-configuration is preferred.

In addition to examining compound (*R*)-**36b** in rats, the pharmacokinetics of the compound were also examined in dogs and monkeys. Compound (*R*)-**36b** exhibited low in vivo clearance with good bioavailability, an acceptable volume of distribution, and a long half-life in the three species examined (Table 10). Although some potential risks associated with long half-life have been discussed,[22] the profile is not critical for further progression of compound (*R*)-**36b**.

On the basis of its potent in vitro activity at TRPV1 and its good pharmacokinetic profile, dienamide (*R*)-**36b** was evaluated in two in vivo models to elucidate the efficacy with regard to the treatment of neuropathic pain. Sciatic nerve constriction injury was produced by the method previously described by Fisher et al.[23] Compound (*R*)-**36b** or vehicle was administered orally to male Sprague−Dawley rats 18−21 days after sciatic nerve constriction, and the reversal of established mechanical allodynia was evaluated with von Frey filaments. The results are shown in Figure 2. Treatment with compound

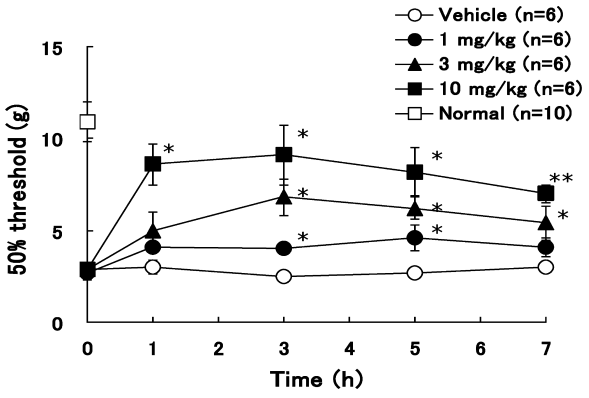

Figure 2. Effect of (*R*)-**36b** on mechanical allodynia in rats with sciatic nerve injury. (*R*)-**36b** was orally administered to rats. Data indicate the mean ± SE: (*) $p < 0.05$, (**) $p < 0.01$ compared with the vehicle-treated group (Steel test).

(*R*)-**36b** significantly blocked the mechanical allodynia in a dose-dependent fashion.

On the basis of this encouraging result, compound (*R*)-**36b** was also evaluated for its ability to block thermal hyperalgesia in rats (Figure 3). In this model, the thermal sensitivity of rats was

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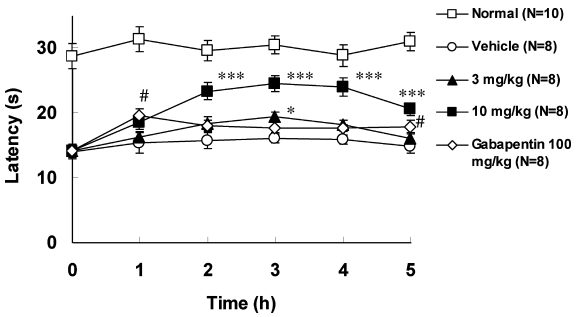

Figure 3. Effect of (*R*)-**36b** on thermal hyperalgesia in rats with sciatic nerve injury. (*R*)-**36b** or gabapentin was orally administered to rats. Data indicate the mean ± SE: (*) $p < 0.05$, (***) $p < 0.001$ (Dunnett test), (#) $p < 0.05$ (Student's *t* test) compared with the vehicle-treated group.

assayed on a hot plate at 48 °C. The hind paw withdrawal latencies were measured hourly following drug and placebo administration. Compound (*R*)-**36b** reversed the thermal hyperalgesia at 3 and 10 mg/kg relative to vehicle-injected controls. The magnitude of the effect at 10 mg/kg was greater than that observed for rats treated with gabapentin (100 mg/kg), a popular drug used to relieve neuropathic pain.

In conclusion, we have developed a novel and potent chemical series of 5,5-diphenylpentadienamides for targeting TRPV1 in vitro and in vivo. Although our initial lead compound **7a**, which was designed from an enamide scaffold that serves as a TRPV1 receptor agonist, was found to be a potent TRPV1 antagonist, many of the properties of the compounds in pentadienamide libraries are not druglike, and their use as pharmaceutical agents is expected to be hampered by their large size, instability, and high lipophilicity. In this investigation, we examined a variety of replacements for the 5-position of dienamides, with the goal of addressing issues related to pharmacokinetics. Our data suggest that substitution with alkoxy groups on the phenyl ring at the 5-position increases the ability of the compounds to penetrate the blood–brain barrier. Furthermore, we found that addition of a 3-hydroxy-2-oxo-1,2,3,4-tetrahydro-5-quinolyl group to the amide moiety gave the best results. This investigation culminated in the discovery of compound (*R*)-**36b**, (2*E*,4*Z*)-*N*-[(3*R*)-3-hydroxy-2-oxo-1,2,3,4-tetrahydro-5-quinolyl]-5-(4-isopropoxyphenyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide, which showed a good pharmacokinetic profile.

In vivo, compound (*R*)-**36b** was found to be effective for preventing mechanical allodynia in rats in a dose-dependent manner, and it reversed thermal hyperalgesia in a model of neuropathic pain induced by sciatic nerve injury. On the basis of its enhanced overall profile compared to other compounds, compound (*R*)-**36b** was chosen as our first clinical candidate TRPV1 antagonist for further evaluation as a potential new treatment for neuropathic pain.

■ EXPERIMENTAL SECTION

General. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. All reactions involving air- or moisture-sensitive reagents were performed under an argon atmosphere. Melting points were determined on a Büchi melting point B-510 apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a JNM-EX270 at 270 MHz or JEOL AL 300 at 300 MHz. Chemical shifts are reported in ppm units with trimethylsilane as the internal standard. Electrospray ionization mass spectra were recorded on a Waters 2795 HPLC, equipped with a Waters 996 photodiode array detector and a Micromass ZMD 2000 LC–MS system. Elemental analyses were performed with an EA 1122 Automatic elemental analyzer, CE Instruments, and were within 0.4% of calculated values. Preparative column chromatography was performed using prepacked silica gel cartridges (YAMAZEN). All final compounds were purified to >95% purity, as determined by reverse-phase high-performance liquid chromatography. Purity was determined by YMC AS-302 column (150 mm × 4.6 mm) at 30 °C with a 1.0 mL/min flow rate using a gradient of 20–90% [0.05% trifluoroacetic acid (TFA) in acetonitrile/water (1:9)] in [0.05% TFA in acetonitrile/water (9:1)] over 15 min.

Ethyl (*E*)-5,5-Dibromo-2,4-pentadienoate (4). To a solution of tetrabromomethane (51.8 g, 156 mmol) in dichloromethane (300 mL) was added triphenylphosphine (90.1 g, 343 mmol). A stirred solution was cooled to 0 °C, and ethyl (*E*)-4-oxo-2-butenoate (**3**) (10.0 g, 78.0 mmol) in dichloromethane (70 mL) was added dropwise. The reaction mixture was stirred at 0 °C for a further 2.5 h. The solution was concentrated under reduced pressure, purified by column chromatography (elution with hexane/ethyl acetate, 5:1), and gave the title compound **4** as a pale-orange solid (17.0 g, 77%). ^{1}H NMR ($CDCl_3$): δ 1.31 (t, J = 7.1 Hz, 3H), 4.23 (q, J = 7.1 Hz, 2H), 6.04 (d, J = 15.2 Hz, 1H), 7.08 (d, J = 10.7 Hz, 1H), 7.29 (dd, J = 10.7, 15.2 Hz, 1H).

Ethyl (*E*)-5,5-Bis(4-trifluoromethylphenyl)-2,4-pentadienoate (5a). A solution of ethyl (*E*)-5,5-dibromo-2,4-pentadienoate (**4**) (8.58 g, 30.0 mmol), 4-trifluoromethylphenylboronic acid (14.3 g, 75 mmol), $Pd(PPh_3)_4$ (1.73 g, 1.50 mmol), sodium carbonate (9.54 g, 90.0 mmol) in dioxane (100 mL), and water (50 mL) was refluxed for 5 h. The mixture was cooled to room temperature and partitioned between ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with water, brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 9:1) and afforded the title compound **5a** as a pale yellow solid (10.1 g, 81%). ^{1}H NMR ($CDCl_3$): δ 1.28 (t, J = 7.2 Hz, 3H), 4.19 (q, J = 7.2 Hz, 2H), 6.15 (d, J = 15.0 Hz, 1H), 6.89 (d, J = 11.6 Hz, 1H), 7.29 (dd, J = 11.6, 15.0 Hz, 1H), 7.33–7.39 (m, 4H), 7.59 (d, J = 8.1 Hz, 2H), 7.71 (d, J = 8.1 Hz, 2H).

(*E*)-5,5-Bis(4-trifluoromethylphenyl)-2,4-pentadienoic Acid (6a). To a solution of ethyl (*E*)-5,5-bis(4-trifluoromethylphenyl)-2,4-pentadienoate (**5a**) (10.1 g, 24.4 mmol) in THF (50 mL) and methanol (50 mL) was added 1.0 M aqueous lithium hydroxide (50 mL). The reaction mixture was stirred at room temperature for 2 h and then concentrated under reduced pressure. The resulting mixture was dissolved in water (1000 mL), acidified with 6 M HCl to pH ∼5, and stirred for a further 1 h. The solid was collected by filtration, dried, and afforded the title compound **6a** as a pale-yellow solid (9.41 g, 100%). ^{1}H NMR ($CDCl_3$): δ 6.14 (d, J = 15.2 Hz, 1H), 6.88 (d, J = 11.6 Hz, 1H), 7.30–7.39 (m, 4H), 7.35 (dd, J = 11.6, 15.2 Hz, 1H), 7.60 (d, J = 8.2 Hz, 2H), 7.72 (d, J = 8.2 Hz, 2H).

(*E*)-*N*-(Isoquinolin-5-yl)-5,5-bis(4-trifluoromethylphenyl)-2,4-pentadienamide (7a). A solution of (*E*)-5,5-bis(4-trifluoromethylphenyl)-2,4-pentadienoic acid (**6a**) (2.32 g, 6.00 mmol), 5-aminoisoquinoline (720 mg, 5.00 mmol), 1- ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (1.92 g, 10.0 mmol), and 1- hydroxybenzotriazole·H_2O (1.15 g, 7.50 mmol) in dimethylformamide (DMF) (30 mL) was stirred at 60 °C for 7 h. The mixture was cooled to room temperature and partitioned between ethyl acetate and saturated aqueous sodium hydrogen carbonate. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 1:1) and afforded the title compound **7a** as a pale yellow solid (1.34 g, 52%), mp 164–166 °C. MS (APCI, positive ion) m/z: 513 (M + 1). ^{1}H NMR ($CDCl_3$): δ 6.38 (d, J = 14.7 Hz, 1H), 6.93 (d, J = 11.6 Hz, 1H), 7.36 (d, J = 8.2 Hz, 2H), 7.39 (d, J = 8.2 Hz, 2H), 7.46 (dd, J = 11.6, 14.7 Hz, 1H), 7.58–7.64 (m, 3H), 7.60 (d, J = 8.2 Hz, 2H), 7.71 (d, J = 8.2 Hz, 2H), 7.83–7.85 (m, 1H), 8.20 (br s, 1H), 8.56–8.58 (m, 1H), 9.27 (s, 1H). Anal. Calcd for $C_{28}H_{18}F_6N_2O$: C, 65.63; H, 3.54; N, 5.47. Found: C, 65.57; H, 3.54; N, 5.20.

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Ethyl (2*E*,4*Z*)-5-Bromo-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (8a). A solution of ethyl (*E*)-5,5-dibromo-2,4-pentadienoate (**4**) (104 mg, 0.367 mmol), 4-trifluoromethylphenylboronic acid (73.1 mg, 0.385 mmol), $Pd(PPh_3)_4$ (21.1 mg, 0.0183 mmol), sodium carbonate (78.3 mg, 0.739 mmol) in THF (1.8 mL), and water (0.73 mL) was stirred at 70 °C for 6 h. The mixture was allowed to cool to room temperature, and water was added. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate twice. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 19:1) and afforded the title compound **8a** as a white solid (84.2 mg, 66%). ^{1}H NMR ($CDCl_3$): δ 1.34 (t, J = 7.1 Hz, 3H), 4.27 (q, J = 7.1 Hz, 2H), 6.20 (d, J = 15.3 Hz, 1H), 7.00 (d, J = 10.8 Hz, 1H), 7.64 (d, J = 8.4 Hz, 2H), 7.70−7.78 (m, 3H).

Ethyl (*E*,*E*)-5-Phenyl-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (9a). A solution of ethyl (2*E*,4*Z*)-5-bromo-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (**8a**) (1.98 g, 5.68 mmol), phenylboronic acid (1.04 g, 8.53 mmol), $Pd_2(dba)_3$ (133 mg, 0.145 mmol), TFP (203 mg, 0.875 mmol), sodium carbonate (1.21 g, 11.4 mmol) in dioxane (28 mL), and water (11 mL) was stirred at 70 °C for 4.5 h. The mixture was allowed to cool to room temperature, and water was added. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate twice. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 19:1) and afforded the title compound **9a** (1.79 g, 91%). ^{1}H NMR ($CDCl_3$): δ 1.27 (t, J = 7.1 Hz, 3H), 4.18 (q, J = 7.1 Hz, 2H), 6.11 (d, J = 15.0 Hz, 1H), 6.82 (d, J = 11.4 Hz, 1H), 7.18−7.21 (m, 2H), 7.38−7.45 (m, 6H), 7.57 (d, J = 8.1 Hz, 2H).

Ethyl (2*E*,4*Z*)-5-(Furan-2-yl)-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (9h). To a mixture of ethyl (2*E*,4*Z*)-5-bromo-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (**8a**) (8.86 g, 25.4 mmol) and $Pd(PPh_3)_4$ (2.94 g, 2.54 mmol) in toluene (177 mL) was added tributyl(2-furyl)tin (18.2 g, 50.9 mmol). The reaction mixture was heated at 100 °C for 5 h, cooled to room temperature, and filtered through Celite. Saturated aqueous aqueous sodium hydrogen carbonate was added to the filtrates. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate twice. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 9:1) and afforded the title compound **9h** (7.44 g, 87%). ^{1}H NMR ($CDCl_3$): δ 1.32 (t, J = 7.2 Hz, 3H), 4.25 (q, J = 7.2 Hz, 2H), 6.08 (d, J = 15.3 Hz, 1H), 6.36 (s, 1H), 6.39 (d, J = 7.2 Hz, 1H), 6.50 (dd, J = 1.8, 3.3 Hz, 1H), 7.49 (d, J = 8.1 Hz, 2H), 7.61−7.65 (m, 3H), 8.18 (dd, J = 11.7, 15.3 Hz, 1H).

(*E*,*E*)-5-Phenyl-5-(4-trifluoromethylphenyl)-2,4-pentadienoic Acid (10a). To a solution of ethyl (*E*,*E*)-5-phenyl-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (**9a**) (1.79 g, 5.18 mmol) in THF (41 mL) and methanol (16 mL) was added 1.1 M aqueous lithium hydroxide (16 mL). The reaction mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure. The resulting mixture was dissolved in water (80 mL), acidified with 1 M HCl (20 mL), and stirred at 0 °C for a further 30 min. The solid was collected by filtration, dried, and afforded the title compound **10a** as a pale-yellow solid (1.63 g, 99%). ^{1}H NMR (DMSO-d_6): δ 6.20 (d, J = 14.7 Hz, 1H), 7.06 (dd, J = 11.5, 14.7 Hz, 1H), 7.17−7.23 (m, 3H), 7.48−7.54 (m, 5H), 7.74 (d, J = 8.4 Hz, 2H).

(*E*,*E*)-*N*-(Isoquinolin-5-yl)-5-phenyl-5-(4-trifluoromethylphenyl)-2,4-pentadinenamide (11a). A solution of (*E*,*E*)-5-phenyl-5-(4-trifluoromethylphenyl)-2,4-pentadienoic acid (**10a**) (1.63 g, 5.12 mmol), 5-aminoisoquinoline (673 mg, 4.66 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl (1.80 g, 9.39 mmol), and 1- hydroxybenzotriazole·H_2O (1.44 g, 9.38 mmol) in DMF (33 mL) was stirred at 60 °C for 11 h. The mixture was allowed to cool to room temperature, and saturated aqueous sodium hydrogen carbonate was added. The organic phase was separated, and the aqueous phase was extracted with ethyl acetate twice. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 3:1). Recrystallization from diethyl ether and hexane afforded the title compound **11a** (742 mg, 36%). MS (ESI, positive ion) m/z: 445 (M + 1). ^{1}H NMR (DMSO-d_6): δ 6.81 (d, J = 13.2 Hz, 1H), 7.16−7.26 (m, 4H), 7.49−7.58 (m, 5H), 7.66 (t, J = 7.9 Hz, 1H), 7.75 (d, J = 8.2 Hz, 2H), 7.94 (d, J = 8.2 Hz, 1H), 7.98 (d, J = 6.0 Hz, 1H), 8.12 (d, J = 7.5 Hz, 1H), 8.55 (d, J = 6.0 Hz, 1H), 9.32 (s, 1H), 10.29 (br s, 1H). Anal. Calcd for $C_{27}H_{19}F_3N_2O$: C, 72.96; H, 4.31; N, 6.30. Found: C, 72.76; H, 4.42; N, 6.12.

Ethyl 3-Phenylpropionate (13). To a stirred solution of phenylacetylene (**12**) (1.00 g, 9.79 mmol) in THF (10 mL) at −78 °C was added dropwise *n*-butyllithium (1.58 M in hexanes, 10.0 mL, 15.8 mmol). The solution was stirred at −78 °C for 45 min, and ethyl chloroformate (1.30 mL, 13.6 mmol) was then added dropwise. After an additional 20 h at −78 °C the reaction was quenched by the dropwise addition of saturated ammonium chloride solution. The solution was extracted with ethyl acetate, and the combined extracts were dried ($MgSO_4$) and evaporated. Purification by column chromatography (elution with hexane/ethyl acetate, 19:1) gave the title compound **13** (1.06 g, 62%). ^{1}H NMR ($CDCl_3$): δ 1.36 (t, J = 7.2 Hz, 3H), 4.30 (q, J = 7.2 Hz, 2H), 7.34−7.48 (m, 3H), 7.57−7.61 (m, 2H).

Ethyl (*Z*)-Iodo-3-phenylacrylate (14). A mixture of ethyl 3-phenylpropionate (**13**) (513 mg, 2.94 mmol) and sodium iodide (1.42 g, 9.48 mmol) in acetic acid (2.2 mL) was heated at 110 °C for 4.5 h. The mixture was cooled and partitioned between ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined extracts were washed with saturated aqueous sodium hydrogen carbonate, sodium thiosulfate, and brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated under reduced pressure to afford the title compound **14** (853 mg, 96%). ^{1}H NMR ($CDCl_3$): δ 6.67 (d, J = 7.9 Hz, 1H), 7.31−7.47 (m, 7H), 7.74 (d, J = 8.0 Hz, 2H), 9.50 (d, J = 8.0 Hz, 1H).

Ethyl (*Z*)-3-Phenyl-3-(4-trifluoromethylphenyl)acrylate (15). A mixture of ethyl (*Z*)-iodo-3-phenylacrylate (**14**) (406 mg, 1.34 mmol), TFP (47.7 mg, 0.205 mmol), 4-trifluoromethylphenylboronic acid (384 mg, 2.02 mmol), $Pd_2(dba)_3$ (31.7 mg, 0.0346 mmol), sodium carbonate (286 mg, 2.70 mmol) in dioxane (6.8 mL), and water (2.7 mL) was heated at 70 °C for 17 h. The mixture was cooled to room temperature and partitioned between ethyl acetate and water. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 19:1) and afforded the title compound **15** (240 mg, 56%). ^{1}H NMR ($CDCl_3$): δ 1.12 (t, J = 6.8 Hz, 3H), 4.06 (q, J = 6.8 Hz, 2H), 6.44 (s, 1H), 7.25−7.41 (m, 7H), 7.65 (d, J = 6.0 Hz, 2H).

(*Z*)-3-Phenyl-3-(4-trifluoromethylphenyl)-2-propen-1-ol (16). To a stirred solution of ethyl (*Z*)-3-phenyl-3-(4-trifluoromethylphenyl)acrylate (**15**) (240 mg, 0.748 mmol) in THF (4.0 mL) at −78 °C was added dropwise a solution of 1.0 M diisobutylaluminum hydride in toluene (2.75 mL, 2.78 mmol). After the solution was stirred at −78 °C for 5 h, the reaction was quenched by the dropwise addition of saturated ammonium chloride solution. The solution was extracted with ethyl acetate, and the combined extracts were washed with brine, dried ($MgSO_4$), and filtered. The filtrate was concentrated under reduced pressure to afford the title compound **16** (201 mg, 97%). ^{1}H NMR ($CDCl_3$): δ 4.20 (d, J = 6.7 Hz, 2H), 6.32 (t, J = 6.7 Hz, 1H), 7.16−7.30 (m, 7H), 7.64 (d, J = 8.0 Hz, 2H).

(*Z*)-3-Phenyl-3-(4-trifluoromethylphenyl)propenal (17). Manganese dioxide (630 mg, 7.25 mmol) was added to a mixture of (*Z*)-3-phenyl-3-(4-trifluoromethylphenyl)-2-propen-1-ol (**16**) (201 mg, 0.723 mmol) in dichloromethane (4.0 mL), and the resulting mixture was stirred at room temperature for 4 h. The mixture was filtered and evaporated to give the title compound **17** (194 mg, 97%). ^{1}H NMR ($CDCl_3$): δ 6.67 (d, J = 7.9 Hz, 1H), 7.31−7.47 (m, 7H), 7.74 (d, J = 8.0 Hz, 2H), 9.50 (d, J = 8.0 Hz, 1H).

Methyl (2*E*,4*Z*)-5-Phenyl-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (18). Methyl (triphenylphosphoranylidene)acetate (290 mg, 0.868 mmol) was added to a mixture of (*Z*)-3-phenyl-3-(4-trifluoromethylphenyl)propenal (**17**) (194 mg, 0.802 mmol) in dichloromethane (2.0 mL). The resulting mixture was stirred at room

temperature for 24 h. Water was added, and the mixture was extracted with ethyl acetate. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 19:1) and afforded the title compound **18** (191 mg, 82%). ^{1}H NMR ($CDCl_3$): δ 3.72 (s, 3H), 6.10 (dd, J = 0.7, 15.2 Hz, 1H), 6.86 (d, J = 11.7 Hz, 1H), 7.23−7.35 (m, 8H), 7.69 (d, J = 8.1 Hz, 2H).

(2*E*,4*Z*)-*N*-(Isoquinolin-5-yl)-5-phenyl-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (20). Following the procedure described for compound **7a**, methyl (2*E*,4*Z*)-5-phenyl-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (**18**) provided the title compound. MS (APCI, positive ion) m/z: 445 (M + 1). ^{1}H NMR (DMSO-d_6): δ 6.79 (d, J = 14.0 Hz, 1H), 7.11 (dd, J = 11.7, 14.0 Hz, 1H), 7.21 (d, J = 11.7 Hz, 1H), 7.33−7.42 (m, 5H), 7.47 (d, J = 8.1 Hz, 2H), 7.66 (t, J = 7.9 Hz, 1H), 7.88 (d, J = 8.1 Hz, 2H), 7.94 (d, J = 8.1 Hz, 1H), 7.97 (d, J = 6.1 Hz, 1H), 8.11 (d, J = 7.4 Hz, 1H), 8.55 (d, J = 5.9 Hz, 1H), 9.32 (s, 1H).

Methyl (*E*)-3-(4-Trifluoromethylphenyl)acrylate (22). Methyl (triphenylphosphoranylidene)acetate (3.66 g, 11.0 mmol) was added to a mixture of 4-trifluoromethylbenzaldehyde (1.52 g, 8.75 mmol) in dichloromethane (15 mL). The resulting mixture was stirred at room temperature for 20 h. Water was added, and the mixture was extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 9:1) and afforded the title compound **22** (1.97 g, 98%). ^{1}H NMR ($CDCl_3$): δ 3.83 (s, 3H), 6.52 (d, J = 15.9 Hz, 1H), 7.61−7.74 (m, 5H).

(2*E*,4*E*)-*N*-(Isoquinolin-5-yl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (27a). Following the procedure described for compound **20**, methyl (*E*)-3-(4-trifluoromethylphenyl)acrylate (**22**) provided the title compound. MS (APCI, positive ion) m/z: 369 (M + 1). ^{1}H NMR (DMSO-d_6): δ 6.73 (d, J = 14.1 Hz, 1H), 7.18 (d, J = 15.0 Hz, 1H), 7.31−7.51 (m, 2H), 7.70 (t, J = 7.8 Hz, 1H), 7.75 (d, J = 8.4 Hz, 2H), 7.84 (d, J = 7.5 Hz, 2H), 7.97 (d, J = 8.4 Hz, 1H), 8.01 (d, J = 6.0 Hz, 1H), 8.19 (d, J = 7.5 Hz, 1H), 8.57 (d, J = 6.3 Hz, 1H), 9.34 (s, 1H), 10.30 (br s, 1H).

Ethyl (2*E*,4*E*)-5-(4-Formylphenyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (28). Analogous to the procedure described for compound **9a**, the title compound **28** was prepared. ^{1}H NMR ($CDCl_3$): δ 1.27 (t, J = 7.1 Hz, 3H), 4.19 (q, J = 7.1 Hz, 2H), 6.16 (d, J = 15.2 Hz, 1H), 6.90 (d, J = 11.4 Hz, 1H), 7.28 (dd, J = 3.7, 11.4 Hz, 1H), 7.34−7.41 (m, 4H), 7.59 (d, J = 8.2 Hz, 2H), 7.97 (d, J = 8.0 Hz, 2H), 10.09 (s, 1H).

(2*E*,4*E*)-5-[4-(Isoquinolin-5-yliminomethyl)phenyl]-*N*-(isoquinolin-5-yl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (30). Analogous to the procedure described for compound **11a**, the title compound **30** was prepared. MS (ESI, positive ion) m/z: 599 (M + 1). ^{1}H NMR (DMSO-d_6): δ 6.87 (d, J = 13.2 Hz, 1H), 7.28 (q, J = 13.5 Hz, 1H), 7.32 (s, 1H), 7.47 (d, J = 8.1 Hz, 2H), 7.57−7.81 (m, 7H), 7.93−8.04 (m, 3H), 8.12−8.15 (m, 2H), 8.23 (d, J = 8.2 Hz, 2H), 8.55 (d, J = 2.7 Hz, 1H), 8.57 (d, J = 2.9 Hz, 1H), 8.86 (s, 1H), 9.34 (d, J = 9.7 Hz, 2H), 10.34 (s, 1H).

(2*E*,4*E*)-5-(4-Formylphenyl)-*N*-(isoquinolin-5-yl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (31). To a stirred solution of (2*E*,4*E*)-5-[4-(isoquinolin-5-yliminomethyl)phenyl]-*N*-(isoquinolin-5-yl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (**30**) (321 mg, 0.536 mmol) in THF (6.4 mL) at room temperature was added hydrochloric acid (1.0 M, 6.4 mL). After the solution was stirred at room temperature for 2 h, the reaction was quenched by the dropwise addition of saturated aqueous sodium hydrogen carbonate. The solution was extracted with ethyl acetate three times, and the combined extracts were dried ($MgSO_4$) and evaporated. Purification by column chromatography (elution with ethyl acetate) gave the title compound **31** (154 mg, 61%). MS (ESI, positive ion) m/z: 473 (M + 1). ^{1}H NMR (DMSO-d_6): δ 6.85 (d, J = 14.6 Hz, 1H), 7.16 (dd, J = 11.5, 14.6 Hz, 1H), 7.33 (d, J = 11.5 Hz, 1H), 7.50 (d, J = 8.1 Hz, 2H), 7.57 (d, J = 8.0 Hz, 2H), 7.66 (t, J = 7.9 Hz, 1H), 7.77 (d, J = 8.4 Hz, 2H), 7.93−7.98 (m, 2H), 8.06 (d, J = 8.0 Hz, 2H), 8.11 (d, J = 7.3 Hz, 1H), 8.55 (d, J = 5.9 Hz, 1H), 9.32 (s, 1H), 10.10 (s, 1H), 10.32 (br s, 1H).

(2*E*,4*E*)-*N*-(Isoquinolin-5-yl)-5-[4-(morpholin-4-ylmethyl)-phenyl]-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (32). To a solution of (2*E*,4*E*)-5-(4-formylphenyl)-*N*-(isoquinolin-5-yl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (**31**) (61.8 mg, 0.131 mmol) in dichloromethane (2.0 mL) were added morpholine (54.7 mg, 0.628 mmol) and sodium triacetoxyborohydride (58.3 mg, 0.275 mmol). After the solution was stirred at room temperature for 6.5 h, the reaction was quenched by the dropwise addition of saturated aqueous sodium hydrogen carbonate. The solution was extracted with chloroform three times, and the combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with chloroform/methanol, 19:1). Recrystallization from diethyl ether afforded the title compound **32** (28.9 mg, 41%). MS (ESI, positive ion) m/z: 544 (M + 1). ^{1}H NMR (DMSO-d_6): δ 2.41−2.42 (m, 4H), 3.55 (s, 2H), 3.60−3.62 (m, 4H), 6.81 (d, J = 13.2 Hz, 1H), 7.19−7.29 (m, 4H), 7.45 (d, J = 7.9 Hz, 2H), 7.56 (d, J = 8.2 Hz, 2H), 7.66 (t, J = 7.9 Hz, 1H), 7.75 (d, J = 8.2 Hz, 2H), 7.93−7.99 (m, 2H), 8.11 (d, J = 6.8 Hz, 1H), 8.55 (d, J = 6.1 Hz, 1H), 9.32 (s, 1H), 10.29 (br s, 1H).

Ethyl (2*E*,4*Z*)-5-(4-Hydroxyphenyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (33). Analogous to the procedure described for compound **9a**, the title compound **33** was prepared. ^{1}H NMR ($CDCl_3$): δ 1.29 (t, J = 7.1 Hz, 3H), 4.21 (q, J = 7.1 Hz, 2H), 5.70 (br s, 1H), 6.10 (d, J = 15.4 Hz, 1H), 6.74 (d, J = 11.5 Hz, 1H), 6.87 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.48 (dd, J = 11.5, 15.4 Hz, 1H), 7.57 (d, J = 8.4 Hz, 2H).

Ethyl (2*E*,4*Z*)-5-(4-Propoxyphenyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (34a). To a stirred solution of ethyl (2*E*,4*Z*)-5-(4-hydroxyphenyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (**33**) (205 mg, 0.566 mmol), 1-propanol (75.3 mg, 1.25 mmol), and triphenylphosphine (306 mg, 1.17 mmol) in toluene (4.1 mL) was added dropwise diethyl azodicarboxylate (2.20 M in toluene, 0.510 mL, 1.12 mmol). After the solution was stirred at room temperature for 13 h, the reaction was quenched by the dropwise addition of saturated aqueous sodium hydrogen carbonate. The solution was extracted with ethyl acetate three times, and the combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. Purification by column chromatography (elution with hexane/ethyl acetate, 8:2) gave the title compound **34a** (204 mg, 89%). ^{1}H NMR ($CDCl_3$): δ 1.07 (t, J = 7.3 Hz, 3H), 1.28 (t, J = 7.1 Hz, 3H), 1.85 (sext, J = 6.6 Hz, 2H), 3.98 (t, J = 6.6 Hz, 2H), 4.19 (q, J = 7.1 Hz, 2H), 6.08 (dd, J = 0.7, 15.3 Hz, 1H), 6.74 (d, J = 11.6 Hz, 1H), 6.94 (dd, J = 2.8, 8.8 Hz, 2H), 7.11 (d, J = 2.8, 8.8 Hz, 2H), 7.41 (d, J = 8.1 Hz, 2H), 7.46 (dd, J = 11.6, 15.3 Hz, 1H), 7.56 (d, J = 8.1 Hz, 2H).

(2*E*,4*Z*)-*N*-(3-Hydroxy-2-oxo-1,2,3,4-tetrahydro-5-quinolyl)-5-(4-propoxyphenyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (36a). Analogous to the procedure described for compound **11a**, the title compound **36a** was prepared, mp 137−138 °C. MS (ESI, positive ion) m/z: 537 (M + 1). ^{1}H NMR (DMSO-d_6): δ 1.00 (t, J = 6.8 Hz, 3H), 1.76 (sext, J = 6.8 Hz, 2H), 2.62 (dd, J = 11.9, 16.0 Hz, 1H), 3.01 (dd, J = 6.2, 16.0 Hz, 1H), 4.00 (t, J = 6.8 Hz, 2H), 4.02−4.06 (m, 1H), 5.44 (br s, 1H), 6.60 (d, J = 14.3 Hz, 1H), 6.69 (dd, J = 3.3, 5.6 Hz, 1H), 7.03−7.19 (m, 8H), 7.53 (d, J = 8.3 Hz, 2H), 7.73 (d, J = 8.3 Hz, 2H), 9.72 (br s, 1H), 10.17 (br s, 1H). Anal. Calcd for $C_{30}H_{27}F_3N_2O_4$: C, 67.16; H, 5.07; N, 5.22. Found: C, 66.95; H, 4.82; N, 5.36.

(2*E*,4*Z*)-5-(4-Cyanomethoxyphenyl)-*N*-(3-hydroxy-2-oxo-1,2,3,4-tetrahydro-5-quinolyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (36g). To a stirred solution of (2*E*,4*Z*)-*N*-(3-hydroxy-2-oxo-1,2,3,4-tetrahydro-5-quinolyl)-5-(4-hydroxyphenyl)-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (**11ab**) (110 mg, 0.222 mmol) and potassium carbonate (34.0 mg, 0.245 mmol) in DMF (2.0 mL) was added chloroacetonitrile (0.0150 mL, 0.245 mmol). After the solution was stirred at room temperature for 3 h, the reaction was quenched by the dropwise addition of water. The solution was extracted with ethyl acetate three times, and the combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. Purification by column chromatography (elution with chloroform/methanol, 5:1) gave the title compound **36g** (57.0 mg, 48%). MS (ESI, positive ion) m/z: 534 (M + 1). ^{1}H NMR (DMSO-d_6): 2.62 (dd, J = 12.0, 15.9 Hz, 1H), 3.02 (dd, J = 6.2, 15.9 Hz, 1H), 4.02−4.11 (m, 1H), 5.25 (s, 2H),

5.44 (d, J = 4.6 Hz, 1H), 6.60−6.72 (m, 2H), 7.09−7.26 (m, 8H), 7.54 (d, J = 8.3 Hz, 2H), 7.74 (d, J = 8.3 Hz, 2H), 9.73 (br s, 1H), 10.16 (br s, 1H).

2-(4-Bromophenoxy)-1,3-diacetoxypropane (38). To a stirred solution of 4-bromophenol (**37**) (5.17 g, 29.9 mmol), diacetin (7.96 g, 45.2 mmol), and triphenylphosphine (11.8 g, 45.1 mmol) in toluene (100 mL) was added dropwise diethyl azodicarboxylate (2.20 M in toluene, 20.0 mL, 44.0 mmol). After the solution was stirred at room temperature for 5.5 h, the reaction mixture was concentrated under reduced pressure. Purification by column chromatography (elution with hexane/ethyl acetate, 7:3) gave the title compound **38** (4.89 g, 49%). ^{1}H NMR ($CDCl_3$): δ 2.07 (s, 6H), 4.28 (d, J = 4.3 Hz, 4H), 5.23−5.27 (m, 1H), 6.88 (td, J = 2.9, 8.2 Hz, 2H), 7.39 (td, J = 2.9, 8.2 Hz, 2H).

2-(4-Bromophenoxy)-1,3-dihydroxypropane (39). To a solution of 2-(4-bromophenoxy)-1,3-diacetoxypropane (**38**) (4.89 g, 14.8 mmol) in THF (118 mL) and methanol (44 mL) was added 2.4 M aqueous lithium hydroxide (44 mL). The reaction mixture was stirred at room temperature for 28 h and then concentrated under reduced pressure. Purification by column chromatography (elution with ethyl acetate) gave the title compound **39** (2.32 g, 64%). ^{1}H NMR ($CDCl_3$): δ 3.87−3.92 (m, 4H), 4.39 (quint, J = 4.3 Hz, 1H), 6.87 (td, J = 3.0, 8.1 Hz, 2H), 7.39 (td, J = 3.0, 8.1 Hz, 2H).

2-(4-Bromophenoxy)-3-tosyloxy-1-propanol (40). To a stirred solution of 2-(4-bromophenoxy)-1,3-dihydroxypropane (**39**) (2.62 g, 10.6 mmol) in THF (50 mL) at 0 °C was added sodium hydride (60% dispersion in oil, 448 mg, 11.2 mmol) and tosyl chloride (2.14 g, 11.2 mmol). After the solution was stirred at 0 °C for 10 min, the reaction was quenched by the dropwise addition of water. The solution was extracted with ethyl acetate three times, and the combined extracts were washed with saturated aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and evaporated. Purification by column chromatography (elution with hexane/ethyl acetate, 1:1) gave the title compound **40** (2.35 g, 55%). ^{1}H NMR ($CDCl_3$): δ 2.45 (s, 3H), 4.11−4.24 (m, 4H), 4.45−4.54 (m, 1H), 6.74 (d, J = 8.1 Hz, 2H), 7.28−7.35 (m, 4H), 7.72 (d, J = 7.2 Hz, 2H).

3-(4-Bromophenoxy)oxetane (41). To a stirred solution of 2-(4-bromophenoxy)-3-tosyloxy-1-propanol (**40**) (470 mg, 1.17 mmol) in THF (19 mL) at 0 °C was added sodium hydride (60% dispersion in oil, 56.3 mg, 1.41 mmol). After the solution was stirred at 40 °C for 23 h, the reaction was quenched by the dropwise addition of saturated aqueous sodium hydrogen carbonate. The solution was extracted with ethyl acetate three times, and the combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. Purification by column chromatography (elution with hexane/ethyl acetate, 7:3) gave the title compound **41** (72.0 mg, 27%). ^{1}H NMR ($CDCl_3$): δ 4.74 (dd, J = 5.2, 6.7 Hz, 2H), 4.93−4.98 (m, 2H), 5.16 (quint, J = 5.2 Hz, 1H), 6.58 (d, J = 8.1 Hz, 2H), 7.38 (d, J = 8.1 Hz, 2H).

Ethyl (2*E*,4*Z*)-5-(4,4,5,5-Tetramethyl[1,3,2]dioxaborolan-2-yl)-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (42). A solution of ethyl (2*E*,4*Z*)-5-bromo-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (**8a**) (167 mg, 0.479 mmol), bis(pinacolato)diboron (147 mg, 0.579 mmol), $Pd(dppf)_2Cl_2$ (19.8 mg, 0.0242 mmol), and potassium acetate (141 mg, 1.44 mmol) in dioxane (3.3 mL) was stirred at 100 °C for 4.5 h. The mixture was allowed to cool to room temperature, and saturated aqueous sodium hydrogen carbonate was added. The residue was extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 9:1) to afford the title compound **42** (76.7 mg, 40%). ^{1}H NMR ($CDCl_3$): δ 1.32 (t, J = 7.4 Hz, 3H), 1.39 (s, 12H), 4.24 (q, J = 7.4 Hz, 2H), 6.06 (d, J = 15.1 Hz, 1H), 7.02 (d, J = 11.7 Hz, 1H), 7.51−7.60 (m, 4H), 8.07 (dd, J = 11.7, 15.1 Hz, 1H).

Ethyl (2*E*,4*Z*)-5-[4-(Oxetan-3-yloxy)phenyl]-5-(4-trifluoromethylphenyl)-2,4-pentadienoate (43). A solution of ethyl (2*E*,4*Z*)-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)- 5-(4-trifluoromethylphenyl)-2,4-pentadienoate (**42**) (424 mg, 1.07 mmol), 3-(4-bromophenoxy)-oxetane (**41**) (244 mg, 1.07 mmol), $Pd(PPh_3)_4$ (186 mg, 0.161 mmol), sodium carbonate (232 mg, 2.19 mmol) in THF (5.3 mL), and water (2.1 mL) was stirred at 70 °C for 6 h. The mixture was allowed to cool to room temperature, and saturated aqueous sodium hydrogen carbonate was added. The residue was extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 6:4) to afford the title compound **43** (246 mg, 55%). ^{1}H NMR (DMSO-d_6): δ 1.28 (t, J = 7.1 Hz, 3H), 4.19 (q, J = 7.1 Hz, 2H), 4.80−4.84 (m, 2H), 4.98−5.03 (m, 2H), 5.26 (quint, J = 5.6 Hz, 1H), 6.09 (d, J = 15.2 Hz, 1H), 6.74 (d, J = 8.6 Hz, 2H), 6.76 (d, J = 11.5 Hz, 1H), 7.12 (d, J = 8.6 Hz, 2H), 7.39 (d, J = 8.6 Hz, 2H), 7.41 (dd, J = 11.5, 15.2 Hz, 1H), 7.57 (d, J = 8.2 Hz, 2H).

(2*E*,4*Z*)-*N*-(3-Hydroxy-2-oxo-1,2,3,4-tetrahydro-5-quinolyl)-5-[4-(oxetan-3-yloxy)phenyl]-5-(4-trifluoromethylphenyl)-2,4-pentadienamide (45). Analogous to the procedure described for compound **11a**, the title compound **45** was prepared. MS (ESI, positive ion) m/z: 551 (M + 1). ^{1}H NMR (DMSO-d_6): δ 2.60 (dd, J = 11.9, 15.9 Hz, 1H), 3.00 (dd, J = 6.3, 15.9 Hz, 1H), 4.01−4.09 (m, 1H), 4.60 (dd, J = 5.5, 7.5 Hz, 2H), 4.92−4.96 (m, 2H), 5.35 (quint, J = 5.5 Hz, 1H), 5.43 (d, J = 4.5 Hz, 1H), 6.59 (d, J = 14.1 Hz, 1H), 6.69 (dd, J = 2.6, 6.5 Hz, 1H), 6.91 (d, J = 8.4 Hz, 2H), 7.05−7.20 (m, 6H), 7.51 (d, J = 8.1 Hz, 2H), 7.72 (d, J = 8.1 Hz, 2H), 9.71 (br s, 1H), 10.16 (br s, 1H).

2-(Dimethylamino)-6-trifluoromethylnicotinic Acid (47a). 2-Chloro-6-trifluoromethylnicotinic acid (**46**) (506 mg, 2.24 mmol) was dissolved in aqueous dimethylamine (50% solution, 1.32 g, 14.7 mmol). After the solution was stirred at room temperature for 25 h, the reaction was quenched by the dropwise addition of 1 M HCl. The solution was extracted with ethyl acetate three times, and the combined extracts were washed with brine, dried ($MgSO_4$), and evaporated to give the title compound **47a** (506 mg, 96%). ^{1}H NMR ($CDCl_3$): δ 3.03 (s, 6H), 7.34 (d, J = 7.9 Hz, 1H), 8.46 (d, J = 7.9 Hz, 1H).

2-(Dimethylamino)-*N*-methoxy-*N*-methyl-6-trifluoromethylnicotinamide (48a). A solution of 2-(dimethylamino)-6-trifluoromethylnicotinic acid (**47a**) (506 mg, 2.16 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (436 mg, 4.47 mmol), 1- ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (842 mg, 4.39 mmol), 1-hydroxybenzotriazole·H_2O (663 mg, 4.33 mmol), and triethylamine (440 mg, 4.35 mmol) in DMF (10 mL) was stirred at room temperature for 14 h. The reaction mixture was diluted with saturated aqueous sodium hydrogen carbonate and extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. Purification by column chromatography (elution with hexane/ethyl acetate, 1:1) gave the title compound **48a** (581 mg, 97%). ^{1}H NMR ($CDCl_3$): δ 3.08 (s, 6H), 3.30 (s, 3H), 3.51 (s, 3H), 6.92 (d, J = 7.5 Hz, 1H), 7.56 (d, J = 7.5 Hz, 1H).

2-(Dimethylamino)-3-(4-fluorobenzoyl)-6-trifluoromethylpyridine (49a). To a stirred solution of 2-(dimethylamino)-*N*-methoxy-*N*-methyl-6-trifluoromethylnicotinamide (**48a**) (581 mg, 2.10 mmol) in THF (12 mL) was added dropwise 4-fluorophenylmagnesium bromide (1.00 M in THF, 10.0 mL, 10.0 mmol). After the solution was stirred at room temperature for 29 h, the reaction was quenched by the dropwise addition of saturated ammonium chloride solution. The solution was extracted with chloroform three times, and the combined extracts were washed with brine, dried ($MgSO_4$), and evaporated. Purification by column chromatography (elution with hexane/ethyl acetate, 9:1) gave the title compound **49a** (479 mg, 73%). ^{1}H NMR ($CDCl_3$): δ 2.95 (s, 6H), 6.97 (d, J = 7.6 Hz, 1H), 7.14−7.20 (m, 2H), 7.65 (d, J = 7.6 Hz, 1H), 7.87−7.92 (m, 2H).

Ethyl (*E*)-3-(2-Dimethylamino-6-trifluoromethylpyridin-3-yl)-3-(4-fluorophenyl)acrylate (50a). To a stirred solution of 2-(dimethylamino)-3-(4-fluorobenzoyl)-6-trifluoromethylpyridine (**49a**) (479 mg, 1.54 mmol) and triethyl phosphonoacetate (3.00 mL, 15.1 mmol) in toluene (20 mL) was added sodium hydride (60%) (623 mg, 15.6 mmol). The solution was stirred at 100 °C for 5 days. The reaction mixture was allowed to cool to room temperature, and water was added. The residue was extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 9:1) to afford the title compound **50a** (125 mg, 21%). ^{1}H NMR ($CDCl_3$): δ 1.15

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(t, J = 7.1 Hz, 3H), 2.88 (s, 6H), 4.11 (q, J = 7.1 Hz, 2H), 6.36 (s, 1H), 7.01−7.06 (m, 3H), 7.24−7.35 (m, 3H).

(*E*)-3-(2-Dimethylamino-6-trifluoromethylpyridin-3-yl)-3-(4-fluorophenyl)-2-propen-1-ol (51a). To a stirred solution of ethyl (*E*)-3-(2-dimethylamino-6-trifluoromethylpyridin-3-yl)-3-(4-fluorophenyl)acrylate (**50a**) (174 mg, 0.456 mmol) in THF (3.4 mL) at −78 °C was added dropwise a solution of 0.99 M diisobutylaluminum hydride in THF (6.00 mL, 5.94 mmol). After the solution was stirred at 0 °C for 7.5 h, the reaction was quenched by the dropwise addition of saturated ammonium chloride solution. The solution was extracted with chloroform three times, and the combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 7:3) to afford the title compound **51a** (121 mg, 78%). ^{1}H NMR ($CDCl_3$): δ 2.90 (s, 6H), 4.05−4.09 (m, 2H), 6.36 (t, J = 7.0 Hz, 1H), 6.99−7.10 (m, 3H), 7.25−7.38 (m, 3H).

(*E*)-3-(2-Dimethylamino-6-trifluoromethylpyridin-3-yl)-3-(4-fluorophenyl)propenal (52a). A solution of (*E*)-3-(2-dimethylamino-6-trifluoromethylpyridin-3-yl)-3-(4-fluorophenyl)-2-propen-1-ol (**51a**) (121 mg, 0.355 mmol) in dichloromethane (2.4 mL) was treated with manganese oxide (573 mg, 6.59 mmol) and stirred at room temperature for 6 h. The solids were removed by filtration, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 7:3) to afford the title compound **52a** (102 mg, 85%). ^{1}H NMR ($CDCl_3$): δ 2.89 (s, 6H), 6.57 (d, J = 7.9 Hz, 1H), 7.07−7.13 (m, 3H), 7.25−7.45 (m, 3H), 9.62 (d, J = 7.9 Hz, 1H).

Methyl (2*E*,4*Z*)-5-(2-Dimethylamino-6-trifluoromethylpyridin-3-yl)-5-(4-fluorophenyl)-2,4-pentadienoate (53a). Methyl (triphenylphosphoranylidene)acetate (129 mg, 0.389 mmol) was added to a mixture of (*E*)-3-(2-dimethylamino-6-trifluoromethylpyridin-3-yl)-3-(4-fluorophenyl)propenal (**52a**) (102 mg, 0.301 mmol) in dichloromethane (2.0 mL). The resulting mixture was stirred at room temperature for 24 h. Water was added, and the mixture was extracted with ethyl acetate three times. The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by column chromatography (elution with hexane/ethyl acetate, 8:2) and afforded the title compound **53a** (110 mg, 92%). ^{1}H NMR ($CDCl_3$): δ 2.84 (s, 6H), 3.73 (s, 3H), 6.09 (d, J = 15.2 Hz, 1H), 6.79 (d, J = 11.5 Hz, 1H), 6.99−7.40 (m, 7H).

(2*E*,4*Z*)-5-(2-Dimethylamino-6-trifluoromethylpyridin-3-yl)-5-(4-fluorophenyl)-*N*-(3-hydroxy-2-oxo-1,2,3,4-tetrahydro-5-quinolyl)-2,4-pentadienamide (55a). Analogous to the procedure described for compound **11a**, the title compound **55a** was prepared. MS (ESI, positive ion) m/z: 541 (M + 1). ^{1}H NMR (DMSO-d_6): δ 2.57−2.66 (m, 1H), 2.97−3.04 (m, 1H), 3.33 (s, 6H), 4.04−4.08 (m, 1H), 5.45 (d, J = 4.2 Hz, 1H), 6.60 (d, J = 12.5 Hz, 1H), 6.68−6.71 (m, 1H), 7.04−7.16 (m, 4H), 7.20−7.29 (m, 4H), 7.35−7.40 (m, 2H), 9.76 (br s, 1H), 10.18 (br s, 1H).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures for synthesis of **7b−f**, **11b−ao**, **27b,c**, **36b−f,h,i**, and **55b**; details of the Ca^{2+} influx functional in vitro assay and in vivo studies. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 81-55-989-4035. Fax: 81-55-986-7430. E-mail: osamu.saku@kyowa-kirin.co.jp.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

TRPV1, transient receptor potential cation channel, subfamily V, member 1; EBNA, Epstein−Barr virus nuclear antigen; TFP, tris(2-furyl)phosphine; NOE, nuclear Overhauser effect; THF, tetrahydrofuran; WSC, water-soluble carbodiimide; FLIPR, fluorometric imaging plate reader; CNS, central nervous system; PBS, phosphate-buffered saline; CYP, cytochrome P450; hERG, human ether-a-go-go-related gene; TFA, trifluoroacetic acid; DMF, dimethylformamide

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