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# Design and Optimization of Benzimidazole-Containing Transient Receptor Potential Melastatin 8 (TRPM8) Antagonists

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Transient receptor potential melastatin 8 (TRPM8) is a nonselective cation channel that is thermoresponsive to cool to cold temperatures (8−28 °C) and also may be activated by chemical agonists such as menthol and icilin. Antagonism of TRPM8 activation is currently under investigation for the treatment of painful conditions related to cold, such as cold allodynia and cold hyperalgesia. The design, synthesis, and optimization of a class of selective TRPM8 antagonists based on a benzimidazole scaffold is described, leading to the identification of compounds that exhibited potent antagonism of TRPM8 in cell-based functional assays for human, rat, and canine TRPM8 channels. Numerous compounds in the series demonstrated excellent in vivo activity in the TRPM8-selective "wet-dog shakes" (WDS) pharmacodynamic model and in the rat chronic constriction injury (CCI)-induced model of neuropathic pain. Taken together, the present results suggest that the in vivo antagonism of TRPM8 constitutes a viable new strategy for treating a variety of disorders associated with cold hypersensitivity, including certain types of neuropathic pain.

## Introduction

Transient receptor potential melastatin 8 (TRPM8[a]) belongs to the melastatin subgroup of the transient receptor potential (TRP) channel superfamily. TRP channels are nonselective cation channels activated by a variety of chemical and physical stimuli. A subset of the TRP channel superfamily is thermoresponsive, each receptor being activated over a discrete temperature range, cumulatively spanning from noxious cold to noxious hot. Specifically, TRPM8 is stimulated by cool to cold temperatures (8−28 °C) and by chemical agonists such as menthol and icilin.[1]

TRPM8 is expressed in a range of tissues predominately in the prostate and liver and, to a lesser degree, in brain, lung, bladder, gastrointestinal tract, blood vessels, and immune cells.[2,3] In addition, TRPM8 has been localized in the dorsal root and trigeminal ganglia to subsets of primary sensory neurons (Aδ and C fibers) which are largely distinct from TRPV1/TRPA1 expressing populations.[1,4] Native and induced expression of TRPM8 on multiple neuronal subtypes provides a rationale for the observed cold hypersensitivity in numerous pathologic conditions.[5] Pain hypersensitivity may manifest in two forms: allodynia, wherein a normally innocuous stimulus triggers a pain response, and hyperalgesia, wherein a noxious stimulus results in a prolonged or more intense response to pain. Allodynia to cold is prevalent in forms of neuropathic pain such as that arising from traumatic neuropathy, or fibromyalgia, whereas cold hyperalgesia is a common manifestation of chronic inflammation, such as occurs in rheumatoid arthritis, although many conditions can present both types of hypersensitivity disorder.[6] Thus, TRPM8 antagonists represent a novel and potentially useful approach to the treatment of these disorders for which there continues to be a high unmet medical need.[7] Indeed, numerous patents[8] and journal articles[9] have recently been disclosed in the literature purporting the utility of TRPM8 antagonists in the treatment of various disease states such as urological disorders, asthma, COPD, prostate and colon cancers, and pain. A diverse range of scaffolds have been employed as TRPM8 antagonists, among them are menthylamides, benzyloxybenzylamides, benzyloxybenzamides, alkylphosphonate esters, arylacetamides, and fused amino-oxazoles and thiazoles.

In this paper, we describe the preparation of heteroatom-substituted spiro[4,5]dec-2-enes substituted at the 2-position of a benzimidazole core (Figure 1) and the lead optimization studies that culminated in the preparation of **5**, an orally bioavailable, potent, and selective TRPM8 antagonist exhibiting antiallodynic properties in in vivo pain models.

## Chemistry

The synthesis of target compounds **1**−**3** is illustrated in Scheme 1. Reduction of the biphenylnitroaniline **6** (vide infra)

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[a] Abbreviations: TRPM8, transient receptor potential melastatin 8; LM, liver microsomes; MTCA, methyl trichloroacetamidate; ECHA, ethyl chlorohydroximinoacetate; MTP, methylenetriphenylphosphorane; DMBB, 3,3-dimethylbenzo[*c*][1,2]oxaborol-1(3*H*)-ol; pyBrOP, bromotripyrrolidinophosphonium hexafluorophosphate; WDS, "wet-dog" shakes; CCI, chronic constriction injury; FLIPR, fluorometric imaging plate reader; FDSS, functional drug screening system; NMR, nuclear magnetic resonance; TMS, tetramethylsilane; TLC, thin layer chromatography; HPLC, high pressure liquid chromatography; CAM, ceric ammonium molybdate; CSA, 10-camphorsulfonic acid.



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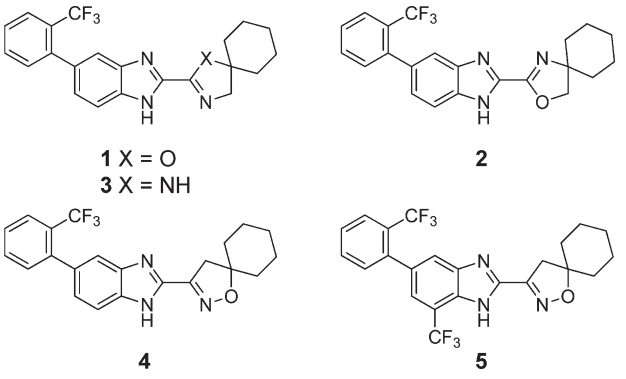
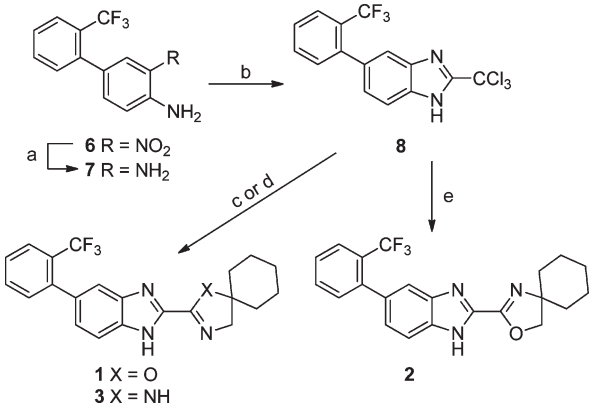



**Figure 1.** Prototypical structures of the spiro-oxazolines (**1** and **2**), spiro-imidazoline (**3**), and spiro-isoxazoline (**4**) substituted benzimidazole ring systems and the structure of optimized TRPM8 antagonist **5**.

**Scheme 1**[a]




[a] Reagents and conditions: (a) $H_2$, 10% Pd on C, EtOH, rt, 15 h; (b) MTCA, AcOH, rt, 14 h; (c) (1-aminomethyl)cyclohexanol hydrochloride, NaOH, $H_2O$, dioxane, rt, 14 h; (d) 1-aminomethyl-1-cyclohexylamine, THF, TEA, rt, 1 h; (e) (1-aminocyclohexyl)methanol, $H_2O$, dioxane, rt, 14 h.

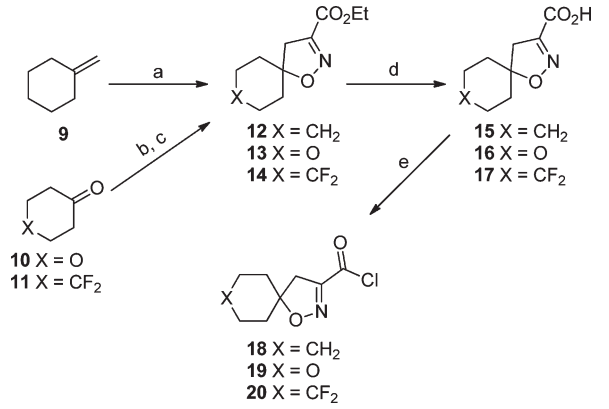
was performed by catalytic hydrogenation to yield biphenyldiamine **7**. Reaction of **7** with methyl trichloroacetamidate (MTCA) afforded 2-trichloromethylbenzimidazole **8** as a key intermediate.[10] Treatment of **8** with the appropriate amino alcohol or diamine produced **1**−**3**.

The spiro-isoxazoline-substituted benzimidazole series was synthesized in a convergent manner through the assembly of the benzimidazole five-membered ring in the final step. The key intermediates for this step were the isoxazoline carboxylic acid chlorides **18**−**20** (Scheme 2) and the biphenylnitroanilines **6**, **34**−**47**, **49** (Schemes 3 and 4).

The spiro-isoxazoline ring system was synthesized via a [3 + 2] dipolar cycloaddition between the in situ generated nitrile oxide and *exo*-methylenecycloalkyl (Scheme 2). Isoxazoline ester **12**[11] was prepared from commercially available ethyl nitroacetate in which the reactive dipolarophile is generated via dehydration, whereas esters **13** and **14** were prepared by the base-induced elimination of HCl from ethyl chlorohydroximinoacetate (ECHA). Saponification of the esters gave the corresponding carboxylic acids **15**−**17**, which were treated with oxalyl chloride to afford the isoxazoline carboxylic acid chlorides **18**−**20**.

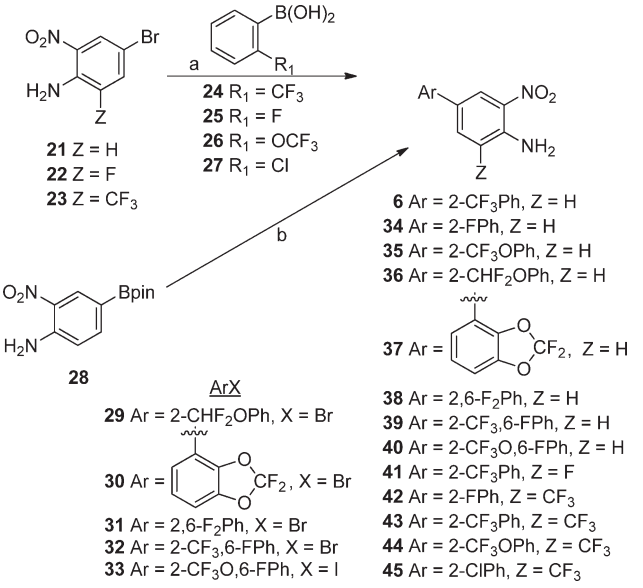
Biphenylnitroanilines **6**, **34**−**45** were prepared by Suzuki coupling from the 4-halo-2-nitroanilines **21**−**23** and 2-substituted phenylboronic acids **24**−**27** (Scheme 3). In some

**Scheme 2**[a]




[a] Reagents and conditions: (a) $NO_2CH_2CO_2Et$, DABCO, EtOH, 80 °C, 42 h; (b) "$Ph_3P{=}CH_2$",[22] $Et_2O$, rt 16 h; (c) ECHA, DIPEA, DCM, rt, 24 h; (d) LiOH, MeOH, $H_2O$, rt, 16 h; (e) $(COCl)_2$, DCM, cat. DMF, rt, 1 h.

**Scheme 3**[a]



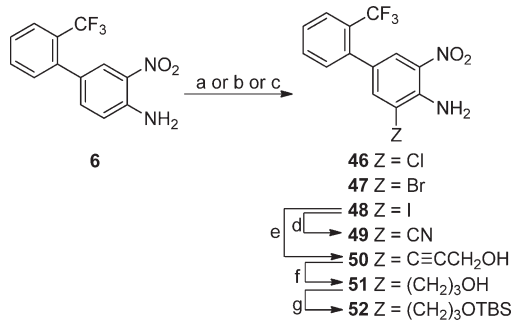

[a] Reagents and conditions: (a) $(dppf)PdCl_2 \cdot DCM$, DME, 2 M aq $Na_2CO_3$, 90 °C; (b) ArX, $(dppf)PdCl_2 \cdot DCM$, DME, 2 M aq $Na_2CO_3$, 90 °C. Bpin = 4,4,5,5-tetramethyl-1,3,2-dioxaborolyl.

instances, the boronic acid or ester of the substituted aryl was not commercially available, so the coupling partners were reversed. In this case, the aminonitrophenylboronate ester **28** was used for the Suzuki coupling with the aryl halides **29** and **30** to afford biphenylnitroanilines **36** and **37**, respectively. This coupling strategy was also employed for the 2,6-disubstituted aryl bromides and iodides **31**−**33** because better yields of biphenylnitroanilines **38**−**40** were obtained.

Biphenylnitroaniline **6** was halogenated according to Scheme 4 to afford **46**−**48**. Treatment of iodide **48** with CuCN afforded the nitrile **49**. Silyl ether **52** was prepared via a Sonagashira coupling reaction between **48** and propargyl alcohol to afford acetylene **50**. Hydrogenation of **50** produced **51**, which was subsequently protected with TBSCl to afford silyl ether **52** (Scheme 4).

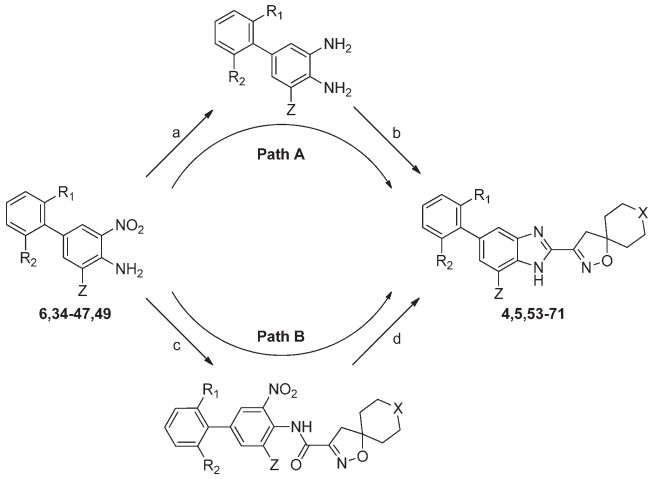
The benzimidazole core was assembled via the two general routes outlined in Scheme 5. In the first method (path A), the biphenylnitroaniline was reduced to the biaryldiamine and used without purification. Acylation with one of the acid chlorides

**Scheme 4**[a]




[a] Reagents and conditions: (a) NCS, ACN, 80 °C, 72 h; (b) $Br_2$, AcOH, rt, 30 min; (c) $I_2$, $Ag_2SO_4$, EtOH, rt, 24 h; (d) CuCN, DMA, 140 °C, 14 h; (e) propargyl alcohol, $(Ph_3P)_2PdCl_2$, CuI, THF, TEA, rt, 16 h; (f) $H_2$, 10% Pd on C, EtOH, rt, 16 h; (g) TBSCl, imidazole, DCM, rt, 2 h.

**Scheme 5**[a]



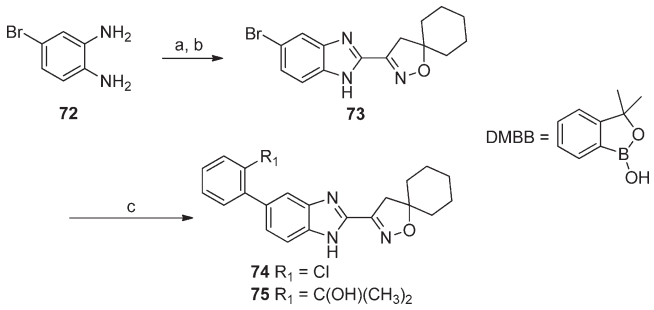

[a] Reagents and conditions: (a) Fe, HCl or $H_2$, 10% Pd on C, EtOH; (b) (i) **18**−**20**, TEA, DCM, rt, (ii) CSA, dioxane, 100 °C; (c) (i) NaH, THF, (ii) **18**−**20**; (d) Fe, AcOH, 80 °C.

**18**−**20** produced a mixture of monoamides and a minor amount of the diacylated byproduct, which was separated from the monoamide mixture prior to cyclization. The production of the diacylated byproduct could be minimized through the use of a slight excess of the biarylamine and employing a slow addition of the acid chloride solution to the reaction mixture. The monoamide mixture was cyclized under acid catalysis to afford the corresponding benzimidazole.

In the alternate synthesis (Scheme 5, path B), the biphenylnitroaniline was deprotonated with the strong base NaH to afford the sodium anilide salt, which was subsequently treated with one of the acid chlorides **18**−**20** to produce the corresponding biphenylnitroamide. Reduction of the nitro group with iron in acetic acid produced the desired benzimidazole in a one-pot reduction/acid-catalyzed cyclization cascade.

The presence of the acid-sensitive tertiary alcohol in benzimidazole **75** necessitated the reversal of the reaction sequence (Scheme 6). Bromobenzimidazole **73** was prepared by acylation of 4-bromobenzene-1,2-diamine (**72**) with **18** followed by acid-catalyzed cyclization. Subsequent Suzuki coupling with 3,3-dimethylbenzo[*c*][1,2]oxaborol-1(3*H*)-ol[12] (DMBB) afforded the desired product **75**. Benzimidazole **74** was also prepared using the same synthetic sequence except using boronic acid **27**.

**Scheme 6**[a]




[a] Reagents and conditions: (a) **18**, TEA, DCM, rt, 1 h; (b) dioxane, CSA, 100 °C, 3 h; (c) DMBB or **27**, (dppf)$PdCl_2$·DCM, DME, 2 M aq $Na_2CO_3$, 90 °C.

## Results and Discussion

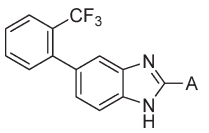
TRPM8 in vitro assays used to assess the functional inhibitory activity of test compounds utilized either a Fluorometric Imaging Plate Reader (FLIPR) manufactured by Molecular Devices or a Functional Drug Screening System (FDSS) manufactured by Hamamatsu. These devices measured icilin-induced increases in intracellular calcium concentration by monitoring changes in fluorescent signal using a $Ca^{2+}$-sensitive fluorescent dye in HEK293 cells that stably or transiently express canine, human, or rat TRPM8 channels.[13] Functional activity of new compounds were assessed in the canine TRPM8 $Ca^{2+}$ flux assay (cTRPM8), and the $IC_{50}$ values obtained or the percent inhibition at a test concentration of 0.2 $\mu$M are shown in Tables 1−4. The metabolic stability of the compounds were assayed in rat, human, and dog liver microsomal (RLM, HLM, and DLM, respectively) preparations and are reported as percent remaining after 10 min of incubation.

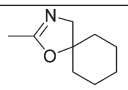
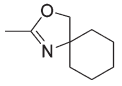
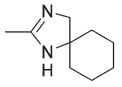
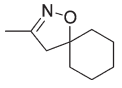
Compounds that were relatively potent in the cTRPM8 functional assay and stable in the liver microsomal preparations were assessed in the "wet-dog" shakes (WDS) pharmacodynamic (PD) model in rats. Rats exhibit shaking and jumping behaviors in response to icilin, a relatively selective and potent TRPM8 agonist.[14] These behaviors are TRPM8-mediated, as they cannot be reproduced in TRPM8-null mice.[5b] Pretreatment of the rats with a TRPM8 antagonist prior to icilin administration inhibits the observed shaking behavior, providing a convenient PD assessment of the test compounds. Thus, compounds were tested at a standard dose of 10 mg/kg, and the percent inhibition of WDS relative to vehicle-treated animals was assessed over time.

Compounds that displayed efficacy in the PD model were evaluated in the rat chronic constriction injury (CCI) model of neuropathic pain. In this model, loose ligatures are surgically placed around the sciatic nerve, resulting in the development of hypersensitivity in the affected limb for up to several weeks.[15] Treatment of the hind paw with cold or substances that evoke cold sensations, such as evaporating acetone, results in exaggerated paw lifting and licking behaviors. Test compounds were orally administered to the rats, and the paw lifting/licking behaviors were monitored over time. The average number of paw lifting/licking events compared to vehicle-treated controls was monitored over time. A reduction in the average number of events (expressed as percent inhibition) is indicative of an antiallodynic effect as a result of the administered compound.

Initially, four potential benzimidazole-containing scaffolds, two oxazoline regioisomers, an imidazoline, and an isoxazoline,

**Table 1.** Functional Activity of the Heteroatom-Substituted Spiro Ring Systems



| Compound | A | cTRPM8 $IC_{50}$ (nM)[a] | Stability[b] HLM/RLM/DLM |
|---|---|---|---|
| **1** |  | 61 | 73/32/40 |
| **2** |  | 6.0 | 85/38/32 |
| **3** |  | 50% | 97/91/36 |
| **4** |  | 3.1 | 100/75/71 |

[a] $IC_{50}$ values or % inhibition at a test concentration of 0.2 $\mu$M averaged from four determinations ($n$ = 4). [b] Percent remaining after 10 min incubations in human (HLM), rat (RLM), or dog (DLM) liver microsomal preparations.

were assessed for functional antagonism of cTRPM8. There was a clear difference in potency between the two oxazoline isomers: oxazoline **2** was 10-fold more potent than oxazoline **1** (Table 1). In addition, the highly polar imidazoline **3** showed poor activity, which may be a consequence of protonation of the basic cyclic amidine functionality (calculated p$K_a$ ca. 9.3).[16] The isoxazoline analogue (**4**) is comparable in potency to oxazoline **2**; however, a distinguishing attribute of **4** was its greater metabolic stability in all three species. Therefore, efforts focused on the isoxazoline tether.

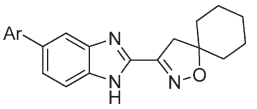
Exploration of the SAR on the isoxazoline scaffold found that substituents in the 2- and 6-positions of the 5-aryl group on the benzimidazole were tolerated (Table 2). Addition of a substituent in the 3-position as in **56** (91 nM) significantly reduced potency compared to the structurally and electronically similar analogues **54** and **55** (1.1 and 2.5 nM, respectively). Hydrophobic substituents at the 2-position generally resulted in single digit nanomolar antagonists of cTRPM8 (**4**, **53**−**55**, and **74**). Furthermore, polar groups such as the tertiary alcohol of **75** were tolerated, however the metabolic stability of **75** was poor.

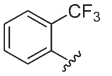
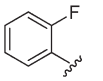
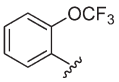
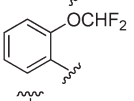
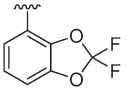
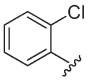
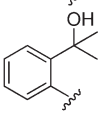
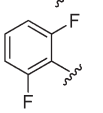
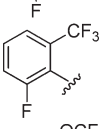
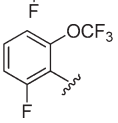
Addition of a second substituent in the 6-position of the 5-phenyl ring has a minor positive effect on the cTRPM8 potency but has a detrimental effect on metabolic stability when direct analogues were compared (**4** to **58**, **53** to **57**, and **54** to **59**). The requirement for an ortho substituent for potency may suggest that the 5-phenyl ring needs to be forced out of planarity with the benzimidazole core for effective binding to the TRPM8 binding site.

To differentiate these potent cTRPM8 antagonists, attention was turned to their metabolic stability. Of these compounds with high metabolic stability across species, **4** was selected to further characterize this emerging family of compounds.

Because the primary screen for the TRPM8 antagonists was conducted using canine TRPM8, the activity of **4** was next assessed in the corresponding rat and human TRPM8 functional assays. The values obtained were 10 and 9 nM, respectively, both similar in potency to the canine value (3.1 nM).

**Table 2.** SAR for Substitution on the 5-Phenyl Ring



| Compound | Ar | cTRPM8 $IC_{50}$ (nM) | Stability[a] HLM/RLM/DLM |
|---|---|---|---|
| **4** |  | 3.1 | 100/75/71 |
| **53** |  | 2.9 | 79/55/47 |
| **54** |  | 1.1 | 100/74/81 |
| **55** |  | 2.5 | 100/59/81 |
| **56** |  | 91 | 100/63/100 |
| **74** |  | 2.4 | 74/45/64 |
| **75** |  | 7.9 | 44/10/4.4 |
| **57** |  | 2.3 | 78/65/62 |
| **58** |  | 0.6 | 38/67/65 |
| **59** |  | 0.8 | 100/44/73 |

[a] Percent remaining after 10 min incubations in human (HLM), rat (RLM), or dog (DLM) liver microsomal preparations.

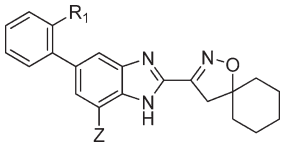
This result can be rationalized on consideration of the high percent identity of the TRPM8 protein sequences across human, rat, and canine species.[13]

Compound **4** exhibited a relatively selective off-target profile when assessed for binding against a panel of 50 GPCRs, transporters, and ion channels (Cerep). Compound **4** showed inhibition of reference compound binding to only two targets by greater than 50% at a concentration of 10 $\mu$M (i.e., 98% at 5-HT1B and 72% at 5-HT2B).

In pharmacokinetic studies in the rat, **4** exhibited relatively high bioavailability (75%), with a modest $C_{max}$ of 230 ng/mL and a long half-life ($t_{1/2}$) of 18 h following an oral dose of 10 mg/kg. The high volume of distribution ($Vd_{ss}$) of 13.6 L/kg (compared to rat total body water volume of 0.688 L/kg) is indicative of extensive tissue distribution and may contribute to the high clearance rate of 54 mL/min/kg, essentially equivalent to hepatic blood flow (55 mL/min/kg).

In the rat PD model, **4** completely inhibited WDS behaviors at a dose of 10 mg/kg po. In the rat CCI time-course study, a dose of 10 mg/kg po of **4** maximally reversed paw lifting/licking behaviors by 66%, peaking after 3 h postdose. The modest oral exposure in rat and potent CYP 2C9 inhibition in HLM

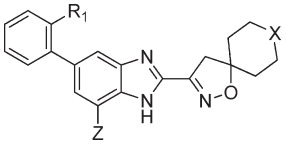
**Table 3.** Functional Activity of Substitution of $R_1$ and Z



| compd | $R_1$ | Z | cTRPM8 $IC_{50}$ (nM) | stability[a] HLM/ RLM/DLM | CYP 2C9 $IC_{50}$ ($\mu$M)[b] |
|---|---|---|---|---|---|
| **4** | $-CF_3$ | $-H$ | 3.1 | 100/75/71 | 1.7 |
| **60** | $-CF_3$ | $-F$ | 2.2 | 46/100/87 | 6.4 |
| **61** | $-CF_3$ | $-Cl$ | 3.3 | 92/100/100 | 6.0 |
| **62** | $-CF_3$ | $-CN$ | 0.7 | 68/96/51 | 1.9 |
| **63** | $-CF_3$ | $-Br$ | 2.7 | 100/83/74 | 2.2 |
| **64** | $-F$ | $-CF_3$ | 1.7 | 71/100/82 | 10 |
| **5** | $-CF_3$ | $-CF_3$ | 0.8 | 92/79/59 | 10 |
| **65** | $-OCF_3$ | $-CF_3$ | 1.2 | 51/53/50 | 10 |
| **66** | $-Cl$ | $-CF_3$ | 1.0 | 66/100/60 | 10 |
| **67** | $-CF_3$ | $-(CH_2)_3OH$ | 0.2 | 57/74/12 | 6.8 |

[a] Percent remaining after 10 min incubations in human (HLM), rat (RLM), or dog (DLM) liver microsomal preparations. [b] $IC_{50}$ values obtained from 15 min HLM incubations with tolbutamide as a substrate.

**Table 4.** TRPM8 Functional Activity with Cyclohexane Modifications



| compd | $R_1$ | Z | X | cTRPM8 $IC_{50}$ (nM) | stability[a] HLM/ RLM/DLM |
|---|---|---|---|---|---|
| **68** | $-CF_3$ | $-H$ | O | 6.7 | 98/74/83 |
| **69** | $-CF_3$ | $-H$ | $CF_2$ | 0.9 | 66/nd[b]/34 |
| **70** | $-CF_3$ | $-CF_3$ | O | 1.4 | 100/96/90 |
| **71** | $-CF_3$ | $-Cl$ | O | 4.0 | 100/82/81 |

[a] Percent remaining after 10 min incubations in human (HLM), rat (RLM), or dog (DLM) liver microsomal preparations. [b] Not determined.

($IC_{50}$ = 1.7 $\mu$M) precluded **4** from further development; however, these liabilities were attenuated in subsequent analogues.

In an attempt to reduce the CYP 2C9 inhibition observed in **4**, modifications to the benzimidazole core were explored and the 7-position was determined to be permissive for substitution (Table 3). In general, hydrophobic substituents (**5**, **60**−**61**, **63**−**66**) and polar groups (**62** and **67**) increased or maintained potency. Acidic functionality was also tolerated, whereas basic moieties resulted in a loss of activity. The most potent compound in this group (**67**) contained a hydroxypropyl group at the 7-position, although it suffered from poor metabolic stability.

In addition, many of the substituents at the 7-position led to a reduction in the HLM CYP 2C9 inhibitory activity. For example, the 7-$CF_3$ substituent resulted in more than a 5-fold decrease in 2C9 inhibition (**64**, **5**, **65**−**67**: $IC_{50}$ ca. 10 $\mu$M) over **4** ($IC_{50}$ = 1.7 $\mu$M). Other substituents that improved CYP 2C9 activity were the 7-fluoro (**60**), 7-chloro (**61**), and 7-hydroxypropyl (**67**).

Another potential site for modification of the scaffold was on the cyclohexyl portion of the spiro-isoxazoline ring system (Table 4). Introduction of an ether oxygen at the 4-position (**68**, **70**, and **71**) resulted in loss of TRPM8 functional activity when compared to the carbocyclic analogues (**4**, **5**, and **61**). The fluorinated derivative **69** increased TRPM8 activity; however, metabolic stability was reduced. Both modifications resulted in an increase of CYP 2C9 inhibition ($IC_{50}$ values of **70** and **71** were 3 $\mu$M or less); thus, these types of modifications were not pursued further.

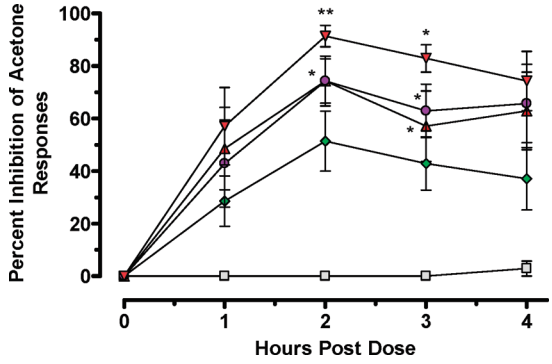



**Figure 2.** Time-course response in the rat CCI cold allodynia model following oral dosing of 10 mg/kg of **5** (red down-pointing triangle), **64** (green diamond), **65** (red up-pointing triangle), and **66** (violet circle) vs vehicle (20% HP$\beta$CD po; gray square). (* $p < 0.05$, ** $p < 0.01$; two-way ANOVA for repeated measures; Bonferroni posthoc test; $n$ = 7 rats/treatment).

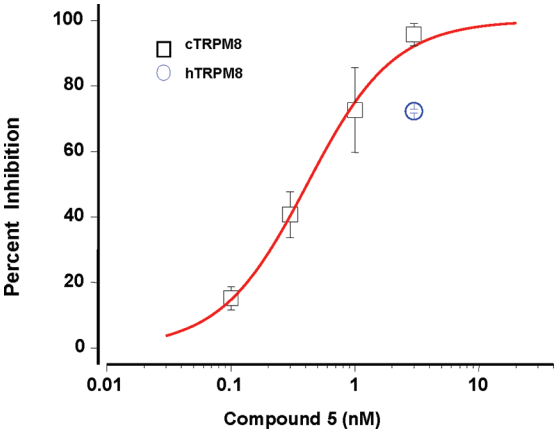



**Figure 3.** Inhibition of cold-stimulated currents in HEK cells expressing canine and human TRPM8. Sequential concentrations of **5** were applied following steady-state current activation achieved by cooling perfusate to 10 °C (from 22 °C).

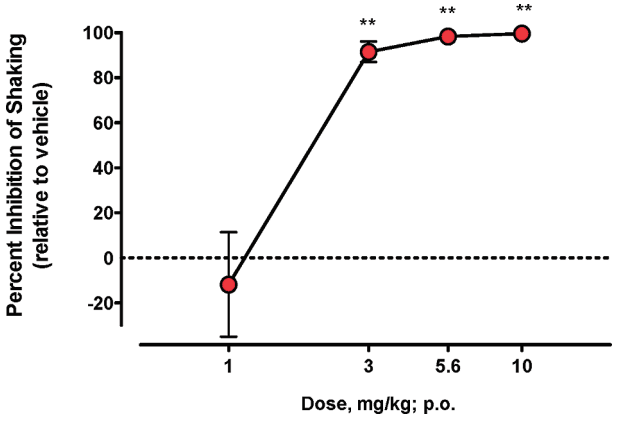



**Figure 4.** Inhibition of icilin-induced (3 mg/kg, ip) WDS in rats following 120 min pretreatment with **5**. (** $p < 0.01$, one-way ANOVA; Dunnett's posthoc test; $n$ = 7 rats/dose).

The potent TRPM8 antagonists **5** and **64**−**66**, which displayed relatively high metabolic stability and the lowest CYP 2C9 inhibitory activities, were further scrutinized in the WDS PD assay. All four compounds completely prevented icilin-induced WDS at oral doses of 10 mg/kg; therefore, the rat CCI cold allodynia model was used as a means to discriminate the compounds. All compounds demonstrated efficacy in the CCI model, albeit to different degrees. At an oral dose of 10 mg/kg, **5** gave the greatest reversal of 91%, whereas **64**−**66** peaked at 51%, 74%, and 74%, respectively, after 2 h postdose (Figure 2).

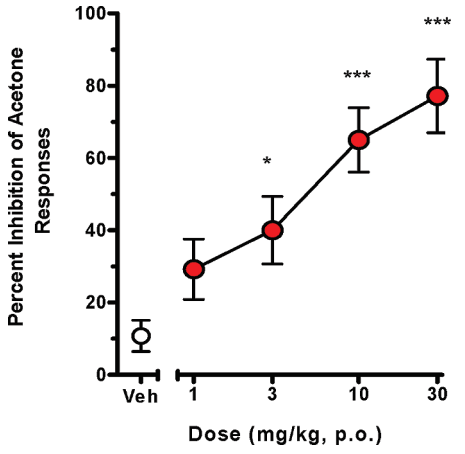



**Figure 5.** Dose−response relationship in the rat CCI cold allodynia model following administration of **5** at 1, 3, 10, or 30 mg/kg, po) assessed 4 h postdose. (* $p < 0.05$; *** $p < 0.001$; two-way ANOVA for repeated measures; Bonferroni posthoc test; $n = 7$−13 rats/treatment).

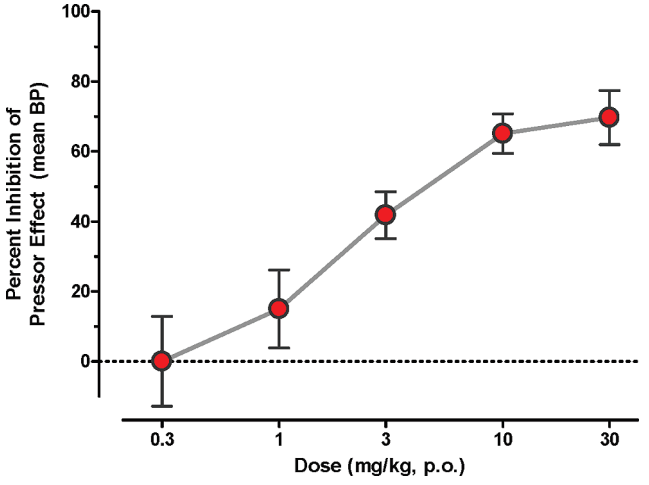



**Figure 6.** Dose−response effects of **5** on the cold-induced increase in mean arterial blood pressure (mmHg) when administered 2 h prior to cold challenge ($n = 7$−11 rats/dose).

Because of its superior efficacy in the CCI pain model, **5** was selected for more detailed studies. In the canine, rat, and human TRPM8 functional assays, **5** exhibited similar potencies, with $IC_{50}$ values of 0.8, 4.0, and 3.0 nM, respectively. In whole cell patch clamp electrophysiology studies, **5** potently inhibited cold-induced TRPM8 currents in HEK293 cells stably expressing canine or human TRPM8, with $IC_{50}$ values of 0.413 and ca. 1 nM, respectively (Figure 3). This inhibition was readily reversed when the compound was removed from the perfusion solution upon washout.

As previously mentioned, **5** completely prevented icilin-induced WDS at an oral dose of 10 mg/kg. In a dose−response study, **5** produced greater than 90% inhibition at 3, 5.6, and 10 mg/kg administered 2 h prior to icilin challenge (Figure 4) and at 150 min postdose, the plasma levels of **5** were 295, 662, and 1042 ng/mL, respectively. The corresponding brain levels of **5** at the 3, 5.6, and 10 mg/kg doses were 175, 414, and 638 ng/g, respectively.

In a dose−response study of the rat CCI cold allodynia model assessed at 4 h postdose, **5** produced an $ED_{50}$ value of 4.5 mg/kg po, with corresponding plasma levels of 254 ng/mL at the 4 h time point (Figure 5). When **5** was administered for 5 consecutive days at 10 mg/kg/day po, no loss of efficacy in reversing CCI cold allodynia was observed between day 1 and day 5, indicating that functional tolerance did not develop under these conditions. It is particularly notable that the in vivo pharmacological profile of **5** faithfully recapitulated the phenotype of TRPM8 null mutant mice in which icilin-induced WDS behavior and CCI-induced cold allodynia are absent.[5b]

The cold pressor test (CPT) can be used to assess efficacy of analgesic compounds[17] as well as to assess cold hypersensitivity.[18] In the present context, it may also be used as a marker of target engagement by a TRPM8 antagonist. Upon cooling, activation of sensory afferent neurons results in blood pressure elevation through sympathetically controlled vasoconstriction. Oral administration of **5** (0.3, 1, 3, 10, or 30 mg/kg) to anesthetized rats 2 h prior to cold pressor testing resulted in a dose-dependent inhibition of the pressor response, suggesting that it blocked the activation of TRPM8 by the cold stimulus (Figure 6).

In PK assessments, benzimidazole **5** had exhibited very high oral bioavailability in the rat (96%) and moderate bioavailability in the dog and monkey (45% and 57%, respectively). Clearance rates in rat (13.9 mL/min/kg) and monkey (15.5 mL/min/kg) were low to moderate and moderate in the dog (14.5 mL/min/kg). Volume of distribution ($Vd_{ss}$) was moderate for the rat (2.92 L/kg) and large for dog (4.43 L/kg) and monkey (4.92 L/kg) in comparison to total body water. Relevant PK parameters are summarized in Table 5.

To obtain an evaluation of its safety potential, the off-target selectivity of **5** was assessed. In cytochrome P450 isoenzyme studies in HLMs, benzimidazole **5** exhibited minimal effect on the enzyme activity of CYP isoforms 3A4, 1A2, 2D6, 2C9, and 2C19 ($IC_{50} > 10\ \mu M$). In a panel of 50 GPCRs, transporters,

**Table 5.** Pharmacokinetic Assessment of **5** in Rat, Dog, And Cynomolgous Monkey after Intravenous and Oral Administration

| | intravenous administration | | | oral administration | | | |
|---|---|---|---|---|---|---|---|
| species | CL (mL/min/kg) | $Vd_{ss}$ (L/kg) | $t_{1/2}$ (h) | $C_{max}$ (ng/mL) | AUC (ng·h/mL) | $t_{1/2}$ (h) | $F$ (%) |
| rat[a] | 13.9 | 2.92 | 3.17 | 1262 | 12302 | 4.74 | 96 |
| dog[b] | 14.5 | 4.43 | nd[c] | 1641 | 5252 | 5.79 | 45 |
| monkey[a] | 15.4 | 4.92 | 5.66 | 510 | 6184 | 4.98 | 57 |

[a] Rats and monkeys were administered 2 mg/kg iv of the HCl salt in 20% HP$\beta$CD or 10 mg/kg po in 20% HP$\beta$CD. [b] Dogs were administered 2 mg/kg iv of the free form in 20% HP$\beta$CD or 10 mg/kg po in 20% HP$\beta$CD. [c] Not determined.

and ion channel binding assays, **5** showed no inhibition of reference compound binding to any of the targets greater than 50% when tested at 10 $\mu$M (Cerep). Similarly, in testing against a panel of 190 kinase assays, **5** did not show inhibition of specific binding for any kinase (Invitrogen).

The cardiovascular safety of **5** was assessed in a whole-cell hERG binding assay, wherein it inhibited [$^3$H]-astemizole binding with an $IC_{50}$ value of 16 $\mu$M. However, when **5** was administered intravenously to anesthetized guinea pigs, no notable hemodynamic or electrocardiographic effects were induced up to a cumulative iv dose of 10 mg/kg. The corresponding plasma level of **5** taken 5 min after the cumulative dose was 18157 ng/mL. No notable effects were observed in a CNS safety assessment study after a single oral dose of 30 or 300 mg/kg of **5** throughout the 14-day observation period.

## Conclusion

In summary, four spirocycle-substituted benzimidazole series were prepared and investigated as TRPM8 antagonists. The spiro-isoxazoline series was selected for optimization due to its superior metabolic stability and potency over the other series. SAR investigations demonstrated that a 2-substituent on the phenyl ring at the 5-position of the benzimidazole core was essential for potency in the TRPM8 in vitro assay, whereas substituents at the 7-position reduced CYP 2C9 inhibition.

Optimization of the spiro-isoxazoline series culminated in the discovery of **5**, a selective, high affinity TRPM8 antagonist that exhibited potent in vitro functional activity and robust oral efficacy in an inflammatory model of neuropathic pain at relatively low plasma levels. Pharmacokinetic data indicate that **5** exhibited moderate to high oral bioavailability across several species, and preliminary pharmacological studies indicate that **5** possesses an appreciable therapeutic window with respect to cardiovascular and CNS safety. Taken together, the present results suggest that the in vivo antagonism of TRPM8 constitutes a viable new strategy for treating a variety of disorders associated with cold hypersensitivity, including certain types of neuropathic pain.

## Experimental Section

**General.** Reagents were purchased from commercial sources and used without further purification. Nuclear magnetic resonance (NMR) spectra were measured in the indicated solvent with tetramethylsilane (TMS) or the residual solvent peak as the internal standard on a Bruker Avance or Varian (300, 400, or 500 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to the internal standard. Abbreviations for the multiplicities of the signals are: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sep (septet), m (multiplet), br (broad), and combinations thereof. Mass spectra (MS) were determined with a Finnegan LCQ Classics or Hewlett-Packard series 1100 mass spectrometer as (ESI) $m/z$ ($M + H^+$) using an electrospray technique. High-resolution mass spectral (HRMS) measurements were obtained using an Agilent LC/MSD TOF which consisted of an 1100 HPLC system coupled with a G1969 MSD TOF mass spectrometer operating in positive electrospray ionization mode.

All reactions were monitored by thin layer chromatography (TLC) carried out on EMD silica gel plates (2.5 cm × 7.5 cm, 250 $\mu$m thick, 60 $F_{254}$), visualized by using UV (254 nm) or stains such as $KMnO_4$, *p*-anisaldehyde, and ceric ammonium molybdate (CAM). All organic solutions were dried over anhydrous $MgSO_4$ or $Na_2SO_4$ and concentrated on a rotary evaporator. Flash chromatography was carried out on an ISCO Combiflash Companion or an Analogix IntelliFlash 280 system using ISCO or Analogix prepacked silica gel columns (12−80 g sizes). High-performance liquid chromatography (HPLC) was carried out on a Varian Prep Star system using a Phenomenex $C_{18}$ (analytical: 10 $\mu$M, 4.6 mm × 150 mm; semiprep: 5 $\mu$M, 21.2 mm × 150 mm) or PrincetonSpher_100 phenyl (analytical: 5 $\mu$M, 4.6 mm × 150 mm; semiprep: 5 $\mu$M, 21.2 mm × 150 mm) reverse phase columns. All compounds used for biological assays are at least of 95% purity based on HPLC analytical results monitored with 220 and 254 nm wavelengths, unless otherwise noted.

**Preparation of the Biphenylnitroanilines 6, 34−45: General Suzuki Procedure.** The boronic acid or ester (1.3 equiv), (dppf)$PdCl_2$·DCM (0.05 equiv), and aryl halide (1 equiv) in a mixture (1.5:1) of DME and 2 M $Na_2CO_3$ (3 equiv) under an argon atmosphere was stirred at 90 °C for 18 h. After cooling to rt, the product was extracted with EtOAc and then the organic solution was dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure, and then the crude product was purified by $SiO_2$ chromatography.

**3-Nitro-2′-(trifluoromethyl)-[1,1′-biphenyl]-4-amine (6).** The title compound was prepared from 4-bromo-2-nitroaniline (**21**) (10.1 g, 46.7 mmol), 2-trifluoromethylphenylboronic acid (**24**) (11.5 g, 60.7 mmol), and (dppf)$PdCl_2$·DCM (1.91 g, 2.34 mmol) in DME (180 mL) and 2 M aq $Na_2CO_3$ (60.0 mL, 120 mmol) according to the general Suzuki procedure, yielding an orange solid (12.8 g, 97%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.11 (d, $J$ = 2.0 Hz, 1H), 7.75 (d, $J$ = 7.8 Hz, 1H), 7.54−7.62 (m, $J$ = 7.6 Hz, 1H), 7.44−7.53 (m, $J$ = 7.8 Hz, 1H), 7.36 (dd, $J$ = 8.6, 1.3 Hz, 1H), 7.33 (d, $J$ = 7.6 Hz, 1H), 6.84 (d, $J$ = 8.6 Hz, 1H).

**2′-Fluoro-3-nitro-[1,1′-biphenyl]-4-amine (34).** The title compound was prepared from nitroaniline **21** (2.17 g, 10.0 mmol), 2-fluorophenylboronic acid (**25**) (1.40 g, 10.0 mmol), and Pd($PPh_3$)$_4$ (116 mg, 0.100 mmol) in dioxane (40 mL) and 2 M aq $NaHCO_3$ (40.0 mL, 80.0 mmol) according to the general Suzuki procedure, yielding a yellow solid (1.80 g, 78%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.36 (d, $J$ = 1.8 Hz, 1H), 7.64 (dt, $J$ = 8.6, 2.0 Hz, 1H), 7.45 (td, $J$ = 7.8, 1.8 Hz, 1H), 7.30−7.38 (m, 1H), 7.13−7.27 (m, 2H), 6.91 (d, $J$ = 8.6 Hz, 1H), 6.18 (br s, 2H).

**3-Nitro-2′-(trifluoromethoxy)-[1,1′-biphenyl]-4-amine (35).** The title compound was prepared from nitroaniline **21** (1.20 g, 5.54 mmol), 2-trifluoromethoxyphenyl-boronic acid (**26**) (1.71 g, 8.31 mmol), and (dppf)$PdCl_2$·DCM (226 mg, 0.277 mmol) in DME (26 mL) and 2 M aq $Na_2CO_3$ (10.0 mL, 20.0 mmol) according to the general Suzuki procedure, yielding an orange solid (1.59 g, 97%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.25 (d, $J$ = 2.0 Hz, 1H), 7.53 (dd, $J$ = 8.7, 2.1 Hz, 1H), 7.39−7.45 (m, 1H), 7.30−7.39 (m, 3H), 6.89 (d, $J$ = 8.6 Hz, 1H), 6.21 (br s, 2H).

**2′-(Difluoromethoxy)-3-nitro-[1,1′-biphenyl]-4-amine (36).** The title compound was prepared from 1-bromo-2-(difluoromethoxy)-benzene (**29**) (223 mg, 1.00 mmol), 4-amino-3-nitrophenylboronic acid (273 mg, 1.50 mmol), and (dppf)$PdCl_2$·DCM (82.0 mg, 0.0100 mmol) in DME (4 mL) and 2 M aq $Na_2CO_3$ (1.50 mL, 3.00 mmol) according to the general Suzuki procedure, yielding an orange solid (291 mg, 100%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.26 (d, $J$ = 2.0 Hz, 1H), 7.58 (dd, $J$ = 8.6, 2.0 Hz, 1H), 7.42 (dd, $J$ = 7.5, 1.9 Hz, 1H), 7.36 (td, $J$ = 7.7, 2.0 Hz, 1H), 7.29 (td, $J$ = 7.5, 1.4 Hz, 1H), 7.22 (dd, $J$ = 8.1, 1.0 Hz, 1H), 6.87 (d, $J$ = 8.6 Hz, 1H), 6.39 (t, $J$ = 73.9 Hz, 1H), 6.16 (br s, 2H).

**4-(2,2-Difluorobenzo[*d*][1,3]dioxol-4-yl)-2-nitroaniline (37).** The title compound was prepared from 4-bromo-2,2-difluorobenzo-[*d*][1,3]dioxole (**30**) (500 mg, 2.11 mmol), 4-amino-3-nitrophenyl-boronic acid pinacol ester (**32**) (724 mg, 2.74 mmol), and (dppf)$PdCl_2$·DCM (86.0 mg, 0.106 mmol) in DME (8 mL) and 2 M aq $Na_2CO_3$ (3.00 mL, 6.00 mmol) according to the general Suzuki procedure, yielding an orange solid (492 mg, 79%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.48 (d, $J$ = 2.3 Hz, 1H), 7.76 (dd, $J$ = 8.8, 2.3 Hz, 1H), 7.26 (dd, $J$ = 8.1, 1.0 Hz, 1H), 7.15 (t, $J$ = 8.1 Hz, 1H), 7.02 (dd, $J$ = 8.0, 1.1 Hz, 1H), 6.93 (d, $J$ = 8.6 Hz, 1H), 6.02 (br s, 2H).

**2′,6′-Difluoro-3-nitro-[1,1′-biphenyl]-4-amine (38).** The title compound was prepared from 1-bromo-2,6-difluorobenzene (**31**) (965 mg, 5.00 mmol), boronate ester **28** (1.72 g, 6.50 mmol), and (dppf)$PdCl_2$·DCM (204 mg, 0.250 mmol) in DME (20 mL) and 2 M aq $Na_2CO_3$ (7.00 mL, 14.0 mmol) according to the general Suzuki procedure, yielding an orange solid (1.14 g, 91%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.29 (s, 1H), 7.46−7.52 (m, 1H), 7.23−7.34 (m, 1H), 6.94−7.04 (m, 2H), 6.90 (d, $J$ = 8.6 Hz, 1H), 6.20 (br s, 2H).

**2′-Fluoro-3-nitro-6′-(trifluoromethyl)-[1,1′-biphenyl]-4-amine (39).** The title compound was prepared from 1-bromo-2-fluoro-6-trifluoromethylbenzene (**32**) (0.850 mL, 6.09 mmol), boronate ester **28** (2.09 g, 7.92 mmol), and (dppf)$PdCl_2$·DCM (249 mg, 0.300 mmol) in DME (24 mL) and 2 M aq $Na_2CO_3$ (8.00 mL, 16.0 mmol) according to the general Suzuki procedure, yielding an orange solid (1.62 g, 89%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.10 (s, 1H), 7.54−7.60 (m, 1H), 7.44−7.53 (m, 1H), 7.35 (t, $J$ = 8.5 Hz, 1H), 7.30 (d, $J$ = 7.3 Hz, 1H), 6.89 (d, $J$ = 8.3 Hz, 1H), 5.44 (br s, 2H).

**2′-Fluoro-3-nitro-6′-(trifluoromethoxy)-[1,1′-biphenyl]-4-amine (40).** The title compound was prepared from 2-fluoro-1-iodo-6-trifluoromethoxybenzene[19] (**33**) (612 mg, 2.00 mmol), boronate ester **28** (687 mg, 2.60 mmol), and (dppf)$PdCl_2$·DCM (81.6 mg, 0.100 mmol) in DME (10 mL) and 2 M aq $Na_2CO_3$ (4.00 mL, 8.00 mmol) according to the general Suzuki procedure, yielding an orange solid (548 mg, 87%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.21 (s, 1H), 7.30−7.47 (m, 2H), 7.18 (d, $J$ = 8.1 Hz, 1H), 7.14 (t, $J$ = 8.6 Hz, 1H), 6.91 (d, $J$ = 6.6 Hz, 1H), 5.37 (br s, 2H).

**3-Fluoro-5-nitro-2′-(trifluoromethyl)-[1,1′-biphenyl]-4-amine (41).** The title compound was prepared from 4-bromo-2-fluoro-6-nitroaniline (**22**) (992 mg, 4.22 mmol), boronic acid **24** (1.04 g, 5.49 mmol), and (dppf)$PdCl_2$·DCM (172 mg, 0.211 mmol) in DME (16 mL) and 2 M aq $Na_2CO_3$ (6.00 mL, 12.0 mmol) according to the general Suzuki procedure, yielding an orange solid (1.12 g, 88%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.93 (t, $J$ = 1.8 Hz, 1H), 7.76 (d, $J$ = 7.8 Hz, 1H), 7.59 (t, $J$ = 7.2 Hz, 1H), 7.51 (t, $J$ = 7.5 Hz, 1H), 7.33 (d, $J$ = 7.6 Hz, 1H), 7.24−7.30 (m, 1H), 6.18 (br s, 2H).

**2′-Fluoro-3-nitro-5-(trifluoromethyl)-[1,1′-biphenyl]-4-amine (42).** The title compound was prepared from 4-bromo-2-nitro-6-trifluoromethyl-phenylamine (**23**) (285 mg, 1.00 mmol), boronic acid **25** (182 mg, 1.30 mmol), and (dppf)$PdCl_2$·DCM (41.0 mg, 0.050 mmol) in DME (4 mL) and 2 M aq $Na_2CO_3$ (1.25 mL, 2.50 mmol) according to the general Suzuki procedure, yielding an orange solid (268 mg, 89%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.49−8.59 (m, 1H), 7.97 (s, 1H), 7.41 (td, $J$ = 7.8, 1.8 Hz, 1H), 7.32−7.39 (m, 1H), 7.23 (td, $J$ = 7.5, 1.1 Hz, 1H), 7.17 (ddd, $J$ = 10.9, 8.2, 1.1 Hz, 1H), 6.75 (br s, 2H).

**3-Nitro-2′,5-bis(trifluoromethyl)-[1,1′-biphenyl]-4-amine (43).** The title compound was prepared from 4-bromo-2-nitro-6-trifluoromethyl-phenylamine (**23**) (10.0 g, 35.2 mmol), boronic acid **24** (8.70 g, 45.8 mmol), and (dppf)$PdCl_2$·DCM (1.44 g, 1.76 mmol) in DME (150 mL) and 2 M aq $Na_2CO_3$ (50.0 mL, 100 mmol) according to the general Suzuki procedure, yielding an orange solid (1.30 g, 92%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.35 (d, $J$ = 2.0 Hz, 1H), 7.78 (d, $J$ = 7.8 Hz, 1H), 7.74 (s, 1H), 7.61 (t, $J$ = 7.2 Hz, 1H), 7.53 (t, $J$ = 7.6 Hz, 1H), 7.33 (d, $J$ = 7.6 Hz, 1H), 6.75 (br s, 2H).

**3-Nitro-2′-(trifluoromethoxy)-5-(trifluoromethyl)-[1,1′-biphenyl]-4-amine (44).** The title compound was prepared from nitroaniline **23** (285 mg, 1.00 mmol), boronic acid **26** (268 mg, 1.30 mmol), and (dppf)$PdCl_2$·DCM (41.0 mg, 0.050 mmol) in DME (4 mL) and 2 M aq $Na_2CO_3$ (1.25 mL, 2.50 mmol) according to the general Suzuki procedure, yielding an orange solid (326 mg, 89%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.50 (d, $J$ = 2.3 Hz, 1H), 7.91 (d, $J$ = 2.0 Hz, 1H), 7.33−7.48 (m, 4H), 6.77 (br s, 2H).

**2′-Chloro-3-nitro-5-(trifluoromethyl)-[1,1′-biphenyl]-4-amine (45).** The title compound was prepared from nitroaniline **23** (1.01 g, 3.55 mmol), 2-chlorophenyl-boronic acid (**27**) (833 mg, 5.33 mmol), and (dppf)$PdCl_2$·DCM (145 mg, 0.178 mmol) in DME (15 mL) and 2 M aq $Na_2CO_3$ (5 mL, 10 mmol) according to the general Suzuki procedure, yielding an orange solid (1.05 g, 94%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.47 (d, $J$ = 2.0 Hz, 1H), 7.89 (d, $J$ = 2.0 Hz, 1H), 7.47−7.53 (m, 1H), 7.30−7.37 (m, 3H), 6.76 (br s, 2H).

**2′-(Trifluoromethyl)-[1,1′-biphenyl]-3,4-diamine (7).** Biphenyl-nitroaniline **6** (818 mg, 2.90 mmol) was dissolved in absolute EtOH (10 mL) then 10% Pd on carbon was added. The reaction was stirred at rt under a $H_2$ atmosphere at balloon pressure for 15 h. The $H_2$ was vented then the suspension was filtered. The solids were washed with MeOH (3 × 10 mL), and then the combined filtrates were concentrated under reduced pressure. The crude product was chromatographed on a 24 g $SiO_2$ prepacked column eluting with 0:1 to 4:1 EtOAc/hexanes, affording the title compound as an off-white solid (570 mg, 78%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.69 (d, $J$ = 8.1 Hz, 1H), 7.49 (t, $J$ = 7.1 Hz, 1H), 7.39 (t, $J$ = 7.5 Hz, 1H), 7.31 (d, $J$ = 7.6 Hz, 1H), 6.64−6.73 (m, 3H), 3.41 (br s, 4H).

**2-(Trichloromethyl)-5-(2-(trifluoromethyl)phenyl)-1*H*-benzimidazole (8).** Biphenyl diamine **7** (222 mg, 0.880 mmol) was dissolved in AcOH (4 mL) then treated with methyl 2,2,2-trichloroacetimidate (MTCA) (0.120 mL, 0.968 mmol) and stirred at rt for 14 h. The reaction was poured into $H_2O$ (50 mL), and the resulting precipitate was isolated by filtration. The solid was washed with $H_2O$ (2 × 10 mL) and then dissolved in EtOAc (50 mL) and dried over $MgSO_4$. The solution was filtered and then concentrated under reduced pressure. The residue was triturated with a minimum amount of DCM, affording the title compound as a yellow powder (326 mg, 100%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 10.46 (br s, 1H), 7.76 (d, $J$ = 7.8 Hz, 1H), 7.57 (t, $J$ = 7.6 Hz, 1H), 7.49 (t, $J$ = 7.6 Hz, 1H), 7.38−7.93 (br s, m, 2H), 7.29−7.38 (m, 2H). Mass spectrum (LCMS, ESI pos.) calcd for $C_{15}H_8Cl_3F_3N_2$ $(M + H)^+$ 379.0, found 379.0.

**2-(5-(2-(Trifluoromethyl)phenyl)-1*H*-benzimidazol-2-yl)-1-oxa-3-azaspiro[4.5]dec-2-ene (1).** 1-(Aminomethyl)cyclohexanol hydrochloride (294 mg, 1.77 mmol) was dissolved in $H_2O$ (8 mL) and treated with 2 M NaOH (0.890 mL, 1.77 mmol), and then a dioxane (16 mL) solution of **8** (163 mg, 0.443 mmol) was added. The reaction was stirred at rt for 14 h and then diluted with brine (30 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was chromatographed on a 24 g $SiO_2$ prepacked column eluting with 0:1 to 2:5 EtOAc/hexanes, affording the title compound (114 mg, 64%). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 7.79 (d, $J$ = 7.8 Hz, 1H), 7.73 (br s, 1H), 7.65 (t, $J$ = 7.2 Hz, 1H), 7.60 (br. s, 1H), 7.56 (t, $J$ = 7.6 Hz, 1H), 7.43 (d, $J$ = 7.6 Hz, 1H), 7.29 (br. d, $J$ = 6.8 Hz, 1H), 3.84 (s, 2H), 1.80−1.98 (m, 4H), 1.69−1.79 (m, 2H), 1.43−1.65 (m, 4H). HRMS calcd for $C_{22}H_{20}F_3N_3O$ $(M + H)^+$ 400.1637, found 400.1630.

**2-(5-(2-(Trifluoromethyl)phenyl)-1*H*-benzo[d]imidazol-2-yl)-3-oxa-1-azaspiro[4.5]dec-1-ene (2).** (1-Aminocyclohexyl)methanol[20] (172 mg, 1.33 mmol) was dissolved in $H_2O$ (8 mL), and then a dioxane (16 mL) solution of **8** (164 mg, 0.445 mmol) was added. The reaction was stirred at rt for 14 h and then diluted with brine (30 mL) and extracted with EtOAc (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was chromatographed on a 24 g $SiO_2$ prepacked column eluting with 0:1 to 2:5 EtOAc/hexanes, affording the title compound (13.9 mg, 7.8%). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 7.79 (d, $J$ = 7.8 Hz, 1H), 7.71 (br s, 1H), 7.65 (t, $J$ = 7.2 Hz, 1H), 7.62 (br s, 1H), 7.56 (t, $J$ = 7.7 Hz, 1H), 7.42 (d, $J$ = 7.6 Hz, 1H), 7.29 (d, $J$ = 6.6 Hz, 1H), 4.32 (s, 2H), 1.75−1.94 (m, 4H), 1.65−1.75 (m, 2H), 1.41−1.65 (m, 4H). HRMS calcd for $C_{22}H_{20}F_3N_3O$ $(M + H)^+$ 400.1637, found 400.1636.

**2-(1,3-Diazaspiro[4.5]dec-2-en-2-yl)-5-(2-(trifluoromethyl)-phenyl)-1*H*-benzimidazole Hydrochloride (3).** A mixture of 1-(aminomethyl)cyclohexylamine dihydrochloride[21] (54.1 mg, 0.269 mmol) and **8** (102 mg, 0.269 mmol) in 8 mL of THF was treated with TEA (0.262 mL, 1.88 mmol) and stirred for 1 h.

The reaction was concentrated under reduced pressure, and then the residue was chromatographed on a 12 g prepacked $SiO_2$ column using 0:1−1:4 EtOH/EtOAc afforded the title compound as a colorless resin (116 mg, quantitative). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.22 (br s, 1H), 7.73 (d, $J$ = 7.8 Hz, 1H), 7.48−7.65 (m, 3H), 7.40−7.48 (m, 1H), 7.33 (d, $J$ = 7.6 Hz, 1H), 7.21 (d, $J$ = 8.6 Hz, 1H), 3.81 (s, 2H), 1.62−1.81 (m, 6H), 1.34−1.53 (m, 4H). Mass spectrum (LCMS, ESI pos.) calcd for $C_{22}H_{21}F_3N_4$ $(M + H)^+$ 399.2, found 399.2.

The title compound (0.269 mmol) dissolved in $Et_2O$ (6 mL) was treated with 1 M HCl in $Et_2O$ (269 $\mu$L, 0.269 mmol). After stirring for 10 min, the precipitate was isolated by filtration and washed with $Et_2O$ (2 × 5 mL). The residual solvent was removed under reduced pressure, affording the hydrochloride salt of the title compound as a white powder (76.6 mg, 65%). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 7.80−7.84 (m, 2H), 7.71 (s, 1H), 7.69 (t, $J$ = 7.3 Hz, 1H), 7.60 (t, $J$ = 7.8 Hz, 1H), 7.39−7.47 (m, 2H), 3.99 (s, 2H), 1.76−2.03 (m, 6H), 1.45−1.69 (m, 4H). HRMS calcd for $C_{22}H_{21}F_3N_4$ $(M + H)^+$ 399.1797, found 399.1795.

**Ethyl 1-Oxa-2-aza-spiro[4.5]dec-2-ene-3-carboxylate (12).** Spiro-isoxazoline ester **12** was prepared according to the literature procedure.[11] To a 300 mL pressure vessel equipped with a magnetic stir bar were added methylenecyclohexane (**9**) (5.83 g, 60.6 mmol) and EtOH (150 mL). Ethyl nitroacetate (16.8 mL, 152 mmol) and DABCO (680 mg, 6.06 mmol) were then added. Additional EtOH (30 mL) was added to rinse the sides of the vessel, and then the vessel was tightly capped. The mixture was heated to 80 °C for 42 h and then cooled to rt. The solvent was removed under reduced pressure, and the residue was divided into three equal portions. Each portion was purified by column chromatography using an 80 g $SiO_2$ prepacked column eluting with 0:1 to 1:4 EtOAc/hexanes, affording the title compound as a colorless liquid (8.12 g, 64%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 4.34 (q, $J$ = 7.2 Hz, 2H), 2.91 (s, 2H), 1.71−1.88 (m, 4H), 1.60−1.71 (m, 2H), 1.39−1.53 (m, 4H), 1.37 (t, $J$ = 7.1 Hz, 3H).

**Preparation of Spiro-isoxazoline Esters 13−14: General Procedure.** Methyl triphenyl phosphonium bromide/sodium amide mixture[22] (MTP) (1.2 equiv, 6.00 mmol) was suspended in dry $Et_2O$ (6 mL) for 1 h at rt, and then an $Et_2O$ (4 mL) solution of the ketone (5.00 mmol) was added. This mixture was stirred at rt for 16 h and then quenched by addition of $H_2O$ (10 mL). The aqueous phase was extracted with $Et_2O$ (2 × 5 mL), and then the organic extracts were combined and dried over $MgSO_4$. DIPEA (1.1 equiv, 5.5 mmol) was added to the dried $Et_2O$ solution (25 mL), and then a DCM (50 mL) solution of ethyl 2-chloro-2-(hydroxyimino)acetate (ECHA) (1 equiv, 5.00 mmol) was added dropwise over 7 h. The mixture was stirred at rt for 60 h, and then the solvent was removed under reduced pressure. The residue was purified by $SiO_2$ chromatography.

**Ethyl 1,8-Dioxa-2-azaspiro[4.5]dec-2-ene-3-carboxylate (13).** The title compound was prepared from MTP (2.50 g, 6.00 mmol), dihydro-2*H*-pyran-4(3*H*)-one (**10**) (0.460 mL, 5.00 mmol), DIPEA (0.96 mL, 5.5 mmol), and ECHA (758 mg, 5.00 mmol) according to the general procedure yielding a tan oil (264 mg, 25%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 4.35 (q, $J$ = 7.2 Hz, 2H), 3.89 (ddd, $J$ = 11.8, 8.7, 3.3 Hz, 2H), 3.65−3.75 (m, 2H), 2.99 (s, 2H), 1.89−1.97 (m, 2H), 1.76−1.86 (m, 2H), 1.37 (t, $J$ = 7.2 Hz, 3H).

**Ethyl 8,8-Difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene-3-carboxylate (14).** The title compound was prepared from MTP (1.95 g, 4.68 mmol), 4,4-difluorocyclohexanone (**11**) (500 mg, 3.73 mmol), DIPEA (0.71 mL, 4.10 mmol), and ECHA (565 mg, 3.73 mmol) according to the general procedure yielding a colorless oil (391 mg, 42%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 4.34 (q, $J$ = 7.2 Hz, 2H), 3.00 (s, 2H), 2.10−2.29 (m, 2H), 1.96−2.09 (m, 4H), 1.81−1.93 (m, 2H), 1.37 (t, $J$ = 7.2 Hz, 3H).

**Preparation of Spiro-isoxazoline Acids 15−17: General Procedure.** The spiro-isoxazoline ester (1 equiv) was dissolved in a mixture of MeOH and $H_2O$ (3:1), and then then $LiOH \cdot H_2O$ (1.1 equiv) was added as a solid. The reaction was stirred at rt for 16 h, and then the solvent was removed under reduced pressure. The residue was dissolved in $H_2O$ and acidified to pH ca. 2 by dropwise addition of 3 M aq HCl. The solution was extracted thrice with DCM, and then the combined organic extracts were dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure.

**1-Oxa-2-azaspiro[4.5]dec-2-ene-3-carboxylic Acid (15).** The title compound was prepared from spiro-isoxazoline ester **12** (1.06 g, 5.00 mmol) and $LiOH \cdot H_2O$ (210 mg, 5.00 mmol) in MeOH (15 mL) and $H_2O$ (5 mL) according to the general procedure, yielding a white powder (766 mg, 92%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 2.93 (s, 2H), 1.72−1.89 (m, 4H), 1.60−1.72 (m, 2H), 1.37−1.54 (m, 4H).

**1,8-Dioxa-2-azaspiro[4.5]dec-2-ene-3-carboxylic Acid (16).** The title compound was prepared from spiro-isoxazoline ester **13** (264 mg, 1.24 mmol) and $LiOH \cdot H_2O$ (57.0 mg, 1.36 mmol) in MeOH (9 mL) and $H_2O$ (3 mL) according to the general procedure, yielding a white powder (228 mg, 99%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.67−3.78 (m, 2H), 3.48−3.58 (m, 2H), 3.01 (s, 2H), 1.69−1.79 (m, $J$ = 5.4, 5.4 Hz, 4H).

**8,8-Difluoro-1-oxa-2-azaspiro[4.5]dec-2-ene-3-carboxylic Acid (17).** The title compound was prepared from spiro-isoxazoline ester **14** (391 mg, 1.58 mmol) and $LiOH \cdot H_2O$ (73.0 mg, 1.74 mmol) in MeOH (12 mL) and $H_2O$ (4 mL) according to the general procedure, yielding a white powder (284 mg, 82%). $^1$H NMR (400 MHz, $CDCl_3$ + $CD_3OD$) $\delta$: 3.00 (s, 2H), 2.10−2.29 (m, 2H), 1.95−2.10 (m, 4H), 1.81−1.95 (m, 2H).

**Preparation of Spiro-isoxazoline Acid Chlorides 18−20: General Procedure.** The spiro-isoxazoline acid (1 equiv) was dissolved in DCM, and then a catalytic amount of DMF (10 $\mu$L) was added. Oxalyl chloride (1.5 equiv) was added dropwise, and then the reaction was stirred at rt for 1 h. The solvent was removed under reduced pressure, and then the residue was taken up in the appropriate solvent as used directly in the next step.

**3-Chloro-5-nitro-2′-(trifluoromethyl)-[1,1′-biphenyl]-4-amine (46).** Biphenyl nitroaniline **6** (12.7 g, 45.0 mmol) was placed in a 250 mL round-bottom flask equipped with a magnetic stir bar and a reflux condenser, and then dry acetonitrile (150 mL) was added. The solid was allowed to dissolve, and then NCS (1.5 equiv, 9.02 g, 67.5 mmol) was added. The reaction was heated at 80 °C for 3 days, cooled to rt, diluted with EtOAc (100 mL), and then washed twice with water (20 mL) and once with brine (30 mL). The combined organic extracts were dried over $MgSO_4$ and filtered, and then the solvent was removed under reduced pressure. The crude material was chromatographed on an 80 g $SiO_2$ prepacked column eluting with 0:1 to 3:7 EtOAc/hexanes, yielding an orange solid (6.96 g, 49%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.10 (d, $J$ = 2.0 Hz, 1H), 7.76 (d, $J$ = 7.6 Hz, 1H), 7.57−7.62 (m, 1H), 7.56 (d, $J$ = 1.8 Hz, 1H), 7.44−7.54 (m, 1H), 7.32 (d, $J$ = 7.3 Hz, 1H), 6.64 (br s, 2H).

**3-Bromo-5-nitro-2′-(trifluoromethyl)-[1,1′-biphenyl]-4-amine (47).** Biphenyl nitroaniline **6** (302 mg, 1.07 mmol) was placed in an 8 mL vial equipped with a magnetic stir bar, and then glacial AcOH (2 mL) was added. The solid was allowed to dissolve, and then $Br_2$ (1.07 equiv, 59.0 $\mu$L, 1.14 mmol) was added dropwise. The reaction was stirred at rt for 30 min, during which time a precipitate formed. The reaction was poured into crushed ice, and the precipitate was isolated by filtration. The precipitate was washed with $H_2O$ (50 mL), and then dissolved in DCM (40 mL). The solution was dried over anhydrous $MgSO_4$ and filtered, and then the solvent removed under reduced pressure. The crude material was chromatographed on a 24 g $SiO_2$ prepacked column eluting with 0:1 to 1:4 EtOAc/hexanes, yielding an orange solid (341 mg, 88%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.14 (d, $J$ = 2.0 Hz, 1H), 7.76 (d, $J$ = 7.8 Hz, 1H), 7.72 (d, $J$ = 2.0 Hz, 1H), 7.59 (t, $J$ = 7.5 Hz, 1H), 7.51 (t, $J$ = 7.7 Hz, 1H), 7.32 (d, $J$ = 7.6 Hz, 1H), 6.70 (br s, 2H).

**3-Iodo-5-nitro-2′-(trifluoromethyl)-[1,1′-biphenyl]-4-amine (48).** Biphenyl nitroaniline **6** (752 mg, 2.66 mmol) was placed in a 40 mL vial equipped with a magnetic stir bar, and anhydrous EtOH (27 mL) was added. Iodine (1.4 equiv, 945 mg, 3.72 mmol) was added as a solid to the stirred solution. Silver sulfate (1.4 equiv,

1.16 g, 3.72 mmol) was added in one portion as a solid, and the reaction was stirred at rt for 24 h. The reaction was filtered, and then the solvent was removed under reduced pressure. The residue was dissolved in DCM (30 mL), washed with 10% aq $Na_2S_2O_3$ (10 mL), dried over anhydrous $MgSO_4$ and filtered, and then the solvent was removed under reduced pressure. The crude product was purified by column chromatography using a 40 g $SiO_2$ prepacked column eluting with 0:1 to 1:4 EtOAc/hexanes, yielding an orange solid (902 mg, 83%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.17 (d, $J$ = 2.0 Hz, 1H), 7.94 (d, $J$ = 2.0 Hz, 1H), 7.76 (d, $J$ = 7.3 Hz, 1H), 7.58 (t, $J$ = 7.6 Hz, 1H), 7.50 (t, $J$ = 7.7 Hz, 1H), 7.32 (d, $J$ = 7.6 Hz, 1H), 6.74 (br s, 2H).

**4-Amino-5-nitro-2′-(trifluoromethyl)-[1,1′-biphenyl]-3-carbonitrile (49).** Biphenyl nitroaniline **48** (230 mg, 0.564 mmol) and Cu(I)CN (1.5 equiv, 7.57 mg, 0.845 mmol) were placed in an 8 mL vial equipped with a magnetic stir bar. Dry dimethylacetamide (2.5 mL) was added, and the vial was tightly capped and was stirred at 140 °C for 14 h. The reaction was cooled to rt and then poured into water. The precipitate was isolated by filtration and washed with water (10 mL). The precipitate was dissolved in EtOAc (25 mL), dried over anhydrous $MgSO_4$, and filtered, and then the solvent was removed under reduced pressure. The crude product was purified by preparative TLC on a 2000 $\mu$m $SiO_2$ plate developed with 1:9 EtOAc/hexanes, yielding a yellow solid (86 mg, 50%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.37 (d, $J$ = 1.8 Hz, 1H), 7.79 (d, $J$ = 7.8 Hz, 1H), 7.71 (d, $J$ = 1.5 Hz, 1H), 7.62 (t, $J$ = 7.3 Hz, 1H), 7.55 (t, $J$ = 7.7 Hz, 1H), 7.31 (d, $J$ = 7.6 Hz, 1H), 6.84 (br s, 2H).

**3-(4-Amino-5-nitro-2′-(trifluoromethyl)-[1,1′-biphenyl]-3-yl)-prop-2-yn-1-ol (50).** Biphenyl nitroaniline **48** (466 mg, 1.14 mmol), $(Ph_3P)_2PdCl_2$ (0.05 equiv, 40.1 mg, 0.057 mmol), and CuI (0.05 equiv, 10.2 mg, 0.054 mmol) were placed in a 40 mL vial equipped with a magnetic stir bar. The vial was evacuated and backflushed with argon, and then anhydrous THF (6 mL) and TEA (4.0 equiv, 0.64 mL, 4.56 mmol) were added. Propargyl alcohol (4 equiv, 0.270 mL, 4.56 mmol) was added, and then the reaction was stirred at rt for 16 h. The solution was diluted with EtOAc (20 mL) and filtered. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography on a 24 g $SiO_2$ prepacked column eluting with 0:1 to 3:2 EtOAc/hexanes, yielding the title compound (279 mg, 73%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 8.10 (d, $J$ = 2.0 Hz, 1H), 7.73 (d, $J$ = 7.6 Hz, 1H), 7.53−7.59 (m, 1H), 7.53 (d, $J$ = 1.8 Hz, 1H), 7.43−7.51 (m, 1H), 7.29 (d, $J$ = 7.6 Hz, 1H), 6.82 (br s, 2H), 4.59 (s, 2H).

**3-(4,5-Diamino-2′-(trifluoromethyl)-[1,1′-biphenyl]-3-yl)-propan-1-ol (51).** Biphenyl nitroaniline **50** (148 mg, 0.440 mmol) was placed in an 8 mL vial equipped with a magnetic stir bar, and then dry EtOH (2 mL) was added. To the ethanol solution was added 10% Pd on activated carbon (27 mg), and then the reaction was stirred under an atmosphere of $H_2$ at balloon pressure for 16 h. The $H_2$ was released, and then the reaction was filtered. The filter cake was washed three times with MeOH (5 mL). The filtrates were combined then the solvent was removed under reduced pressure to afford the title compound (101 mg, 73%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.68 (d, $J$ = 7.6 Hz, 1H), 7.48 (t, $J$ = 7.3 Hz, 1H), 7.38 (t, $J$ = 7.6 Hz, 1H), 7.30 (d, $J$ = 7.6 Hz, 1H), 6.61 (s, 1H), 6.60 (s, 1H), 3.65 (t, $J$ = 5.9 Hz, 2H), 3.48 (br s, 4H), 2.66 (t, $J$ = 7.3 Hz, 2H), 1.86 (quin, $J$ = 6.6 Hz, 2H).

**5-(3-((*tert*-Butyldimethylsilyl)oxy)propyl)-2′-(trifluoromethyl)-[1,1′-biphenyl]-3,4-diamine (52).** Biphenyldiamine **51** (101 mg, 0.324 mmol) was dissolved in DCM (2 mL), and then imidazole (1.1 equiv, 24.5 mg, 0.356 mmol) and TBSCl (1.1 equiv, 53.7 mg, 0.356 mmol) were added sequentially. The reaction was stirred at rt for 2 h. The reaction was filtered, the precipitate was washed once with DCM (5 mL), and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography on a 24 g $SiO_2$ prepacked column eluting with 0:1 to 1:1 EtOAc/hexanes, yielding the title compound (93.2 mg, 68%). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.69 (d, $J$ = 7.8 Hz, 1H), 7.49 (t, $J$ = 7.5 Hz, 1H), 7.38 (t, $J$ = 7.6 Hz, 1H), 7.32 (d, $J$ = 7.6 Hz, 1H), 6.61 (s, 1H), 6.59 (s, 1H), 3.66 (t, $J$ = 5.9 Hz, 2H), 3.52 (br s, 4H), 2.65 (t, $J$ = 7.3 Hz, 2H), 1.75−1.87 (m, 2H), 0.91 (s, 9H), 0.07 (s, 6H).

**Preparation of the Spiro-isoxazoline Benzimidazoles 4, 5, 53−69: General Procedure.** The biphenylnitroaniline (1 equiv) was dissolved in a mixture of EtOH and 3 M HCl (5:1), and then Fe powder (5 equiv) was added and the reaction was stirred at 80 °C for 4 h. The reaction was cooled to rt and filtered. The solids were washed with MeOH, and then the combined filtrates were concentrated under reduced pressure. To the residue was added 2 M NaOH (10 mL), and then the aqueous mixture was extracted with EtOAc (3 × 15 mL). The organic extracts were combined, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure to afford the biphenyldiamine, which was used without purification in the next step.

The biphenyldiamine (1.12 equiv) was dissolved in DCM, and then TEA (3 equiv) was added. A DCM solution of the spiro-isoxazoline acid chloride was added dropwise over 10 min, and then the reaction was stirred at rt for 2 h. The solvent was removed under reduced pressure, and then the residue was purified by column chromatography to afford a mixture of the two monoamides.

The monoamide mixture was dissolved in dry dioxane, and then CSA (0.2 equiv) was added and the reaction was stirred at 100 °C for 4 h. The reaction was cooled to rt and then poured into a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc (3×). The combined organic extracts were dried over $MgSO_4$ and filtered, and then the solvent was removed under reduced pressure. The residue was purified by chromatography to afford the desired benzimidazole.

Optionally, a salt form of the benzimidazole was prepared using the following general procedure: The benzimidazole was dissolved in EtOAc, and then 1 M HCl in $Et_2O$ (1 equiv) was added. A precipitate immediately formed, which was isolated by filtration and rinsed once with EtOAc. The residual solvent was removed under high vacuum to afford the benzimidazole hydrochloride salt.

**3-[5-(2-Trifluoromethyl-phenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene (4).** The title compound was prepared according to the general procedure from biphenyldiamine **7** (157 mg, 0.626 mmol) and TEA (1.00 mL, 7.17 mmol) dissolved in DCM (20 mL) by slow addition of acid chloride **18** (0.796 mmol) in DCM (30 mL), affording a mixture of the monoamides (172 mg, 79%). The monoamide mixture (172 mg, 0.413 mmol) in dioxane (6 mL) was treated with $TsOH \cdot H_2O$ (78.5 mg, 0.413 mmol) at 80 °C for 14 h, yielding a white foam (67.8 mg, 41%). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 7.79 (d, $J$ = 7.8 Hz, 1H), 7.65 (td, $J$ = 7.6, 0.8 Hz, 1H), 7.64 (br s, 1H), 7.56 (t, $J$ = 7.6 Hz, 1H), 7.54 (br s, 1H), 7.43 (d, $J$ = 7.6 Hz, 1H), 7.25 (d, $J$ = 8.1 Hz, 1H), 3.29 (s, 2H), 1.71−1.91 (m, 6H), 1.48−1.64 (m, 4H). HRMS calcd for $C_{22}H_{20}F_3N_3O$ $(M + H)^+$ 400.1637, found 400.1629.

**3-[7-Trifluoromethyl-5-(2-trifluoromethyl-phenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (5).** The title compound was prepared according to the general procedure from nitroaniline **43** (330 mg, 0.942 mmol) and Fe powder (263 mg, 4.71 mmol) in EtOH (5 mL) and 3 M HCl (1 mL) in the first step, and then the resulting biphenyldiamine (0.942 mmol) and TEA (0.330 mL, 2.39 mmol) dissolved in DCM (50 mL) was treated with acid chloride **18** (0.796 mmol) in DCM (30 mL), affording a mixture of the monoamides (272 mg, 70%). The monoamide mixture (272 mg, 0.560 mmol) in dioxane (10 mL) was treated with CSA (26.0 mg, 0.112 mmol) at 100 °C for 3 h, yielding a white foam (255 mg, 97%). Treatment of the benzimidazole (255 mg, 0.545 mmol) in EtOAc (3 mL) with 1 M HCl in $Et_2O$ (0.55 mL, 0.550 mmol) afforded the title compound as a white powder (241 mg, 88%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.89 (d, $J$ = 7.6 Hz, 1H), 7.74−7.81 (m, 1H), 7.71 (s, 1H), 7.65−7.70 (m, 1H), 7.56 (d, $J$ = 7.6 Hz, 1H), 7.48 (s, 1H), 3.33 (s, 2H), 1.63−1.82 (m, 6H), 1.35−1.55 (m, 4H). HRMS calcd for $C_{23}H_{19}F_6N_3O$ $(M + H)^+$ 468.1511, found 468.1515.

**3-[5-(2-Fluorophenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro-[4.5]dec-2-ene Trifluoroacetic Acid Salt (53).** The title compound was prepared according to the general procedure from nitroaniline **34** (175 mg, 0.753 mmol) and Fe powder (210 mg, 3.76 mmol) in EtOH (5 mL) and 3 M HCl (1.5 mL) in the first step, and then the resulting biphenyldiamine (0.942 mmol) and TEA (0.280 mL, 2.03 mmol) dissolved in DCM (30 mL) was treated with acid chloride **18** (0.676 mmol) in DCM (20 mL), affording a mixture of the monoamides (199 mg, 80%). The monoamide mixture (199 mg, 0.543 mmol) in dioxane (6 mL) was treated with CSA (25.0 mg, 0.109 mmol) at 100 °C for 4 h. The product was further purified by HPLC, affording the title compound as the trifluoroacetic acid salt (122 mg, 48%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.76 (s, 1H), 7.71 (d, $J$ = 8.3 Hz, 1H), 7.59 (td, $J$ = 8.0, 1.5 Hz, 1H), 7.47−7.51 (m, 1H), 7.38−7.47 (m, 1H), 7.27−7.38 (m, 2H), 3.30 (s, 2H), 1.63−1.80 (m, 6H), 1.34−1.56 (m, 4H). HRMS calcd for $C_{21}H_{20}FN_3O$ (M + H)$^+$ 350.1669, found 350.1672.

**3-[5-(2-Trifluoromethoxyphenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (54).** The title compound was prepared according to the general procedure from nitroaniline **35** (299 mg, 1.00 mmol) and Fe powder (279 mg, 5.00 mmol) in EtOH (5 mL) and 3 M HCl (1 mL) in the first step, and then the resulting biphenyldiamine (0.932 mmol) and TEA (0.350 mL, 2.50 mmol) dissolved in DCM (50 mL) was treated with acid chloride **18** (0.832 mmol) in DCM (10 mL), affording a mixture of the monoamides (339 mg, 94%). The monoamide mixture (339 mg, 0.783 mmol) in dioxane (6 mL) was treated with CSA (36.0 mg, 0.157 mmol) at 100 °C for 4 h, yielding a white foam (146 mg, 45%). Treatment of the benzimidazole (125 mg, 0.301 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.30 mL, 0.300 mmol) afforded the title compound as a white powder (124 mg, 91%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.72 (d, $J$ = 8.6 Hz, 1H), 7.70 (s, 1H), 7.57−7.63 (m, 1H), 7.47−7.56 (m, 3H), 7.42 (dd, $J$ = 8.3, 1.3 Hz, 1H), 3.31 (s, 2H), 1.62−1.80 (m, 6H), 1.34−1.57 (m, 4H). HRMS calcd for $C_{22}H_{20}F_3N_3O_2$ (M + H)$^+$ 416.1586, found 416.1586.

**3-[5-(2-Difluoromethoxyphenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (55).** The title compound was prepared according to the general procedure from nitroaniline **36** (163 mg, 0.583 mmol) and Fe powder (163 mg, 2.92 mmol) in EtOH (3 mL) and 3 M HCl (0.6 mL) in the first step, and then the resulting biphenyldiamine (0.561 mmol) and TEA (0.210 mL, 1.53 mmol) dissolved in DCM (30 mL) was treated with acid chloride **18** (0.510 mmol) in DCM (20 mL), affording a mixture of the monoamides (163 mg, 77%). The monoamide mixture (163 mg, 0.392 mmol) in dioxane (2 mL) was treated with CSA (18.0 mg, 0.078 mmol) at 100 °C for 3 h, yielding a white foam (80.4 mg, 52%). Treatment of the benzimidazole (80.4 mg, 0.202 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.200 mL, 0.200 mmol) afforded the title compound as a white powder (78.7 mg, 89%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.72 (d, $J$ = 1.0 Hz, 1H), 7.71 (d, $J$ = 5.3 Hz, 1H), 7.48−7.55 (m, 1H), 7.47 (dd, $J$ = 5.6, 1.8 Hz, 1H), 7.45 (dd, $J$ = 6.1, 1.5 Hz, 1H), 7.37−7.41 (m, 1H), 7.33 (d, $J$ = 8.3 Hz, 1H), 7.16 (t, $J$ = 74 Hz, 1H), 3.32 (s, 2H), 1.63−1.83 (m, 6H), 1.37−1.56 (m, 4H). HRMS calcd for $C_{22}H_{21}F_2N_3O_2$ (M + H)$^+$ 398.1680, found 398.1679.

**3-[5-(2,2-Difluorobenzo[1,3]dioxol-4-yl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (56).** The title compound was prepared according to the general procedure from nitroaniline **37** (217 mg, 0.738 mmol) and Fe powder (206 mg, 3.69 mmol) in EtOH (4 mL) and 3 M HCl (0.8 mL) in the first step, and then the resulting biphenyldiamine (0.561 mmol) and TEA (0.210 mL, 1.53 mmol) dissolved in DCM (30 mL) was treated with acid chloride **18** (0.510 mmol) in DCM (20 mL), affording a mixture of the monoamides (172 mg, 79%). The monoamide mixture (172 mg, 0.399 mmol) in dioxane (2 mL) was treated with CSA (19.0 mg, 0.080 mmol) at 100 °C for 3 h, yielding a white foam (120 mg, 73%). Treatment of the benzimidazole (120 mg, 0.291 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.290 mL, 0.290 mmol) afforded the title compound as a white powder (111 mg, 85%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.95 (d, $J$ = 1.0 Hz, 1H), 7.76 (d, $J$ = 8.6 Hz, 1H), 7.67 (dd, $J$ = 8.5, 1.6 Hz, 1H), 7.55 (dd, $J$ = 8.0, 1.1 Hz, 1H), 7.41 (dd, $J$ = 8.1, 1.3 Hz, 1H), 7.34 (t, $J$ = 8.1 Hz, 1H), 1.63−1.82 (m, 6H), 1.37−1.55 (m, 4H). HRMS calcd for $C_{22}H_{19}F_2N_3O_3$ (M + H)$^+$ 412.1473, found 412.1486.

**3-[5-(2,6-Difluorophenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Trifluoroacetic Acid Salt (57).** The title compound was prepared according to the general procedure from nitroaniline **38** (204 mg, 0.815 mmol) and Fe powder (228 mg, 4.08 mmol) in EtOH (5 mL) and 3 M HCl (1.5 mL) in the first step, and then the resulting biphenyldiamine (0.762 mmol) and TEA (0.290 mL, 2.08 mmol) dissolved in DCM (30 mL) was treated with acid chloride **18** (0.508 mmol) in DCM (20 mL), affording a mixture of the monoamides (154 mg, 79%). The monoamide mixture (154 mg, 0.400 mmol) in dioxane (4 mL) was treated with CSA (19.0 mg, 0.080 mmol) at 100 °C for 4 h. The product was further purified by HPLC, affording the title compound as the trifluoroacetic acid salt (121 mg, 63%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.70 (d, $J$ = 8.3 Hz, 1H), 7.66 (s, 1H), 7.43−7.54 (m, 1H), 7.33 (dd, $J$ = 8.6, 1.3 Hz, 1H), 7.19−7.29 (m, 2H), 3.30 (s, 2H), 1.63−1.79 (m, 6H), 1.35−1.55 (m, 4H). HRMS calcd for $C_{21}H_{19}F_2N_3O$ (M + H)$^+$ 368.1574, found 368.1590.

**3-[5-(2-Fluoro-6-trifluoromethylphenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene (58).** The title compound was prepared according to a modified general procedure from nitroaniline **39** (754 mg, 2.51 mmol), $NH_4Cl$ (1.34 g, 25.1 mmol), and Fe powder (701 mg, 12.6 mmol) in EtOH (20 mL) and $H_2O$ (5 mL) stirred at 80 °C for 14 h, affording the biphenyldiamine (662 mg, 98%) in the first step, and then the resulting biphenyldiamine (258 mg, 0.953 mmol) and TEA (0.360 mL, 2.55 mmol) dissolved in DCM (50 mL) was treated with acid chloride **18** (0.851 mmol) in DCM (10 mL), affording a mixture of the monoamides (315 mg, 85%). The monoamide mixture (315 mg, 0.723 mmol) in dioxane (6 mL) was treated with CSA (34.0 mg, 0.145 mmol) at 100 °C for 4 h, yielding a white foam (151 mg, 50%). $^1$H NMR (400 MHz, DMSO-$d_6$ + TFA-$d_1$) $\delta$: 7.63−7.77 (m, 4H), 7.57 (s, 1H), 7.23 (d, $J$ = 8.3 Hz, 1H), 3.31 (s, 2H), 1.63−1.85 (m, 6H), 1.34−1.59 (m, 4H). HRMS calculated for $C_{22}H_{19}F_4N_3O$ (M + H)$^+$ 418.1543, found 418.1552.

**3-[5-(2-Fluoro-6-trifluoromethoxyphenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (59).** The title compound was prepared according to the general procedure from nitroaniline **40** (285 mg, 0.901 mmol) and Fe powder (252 mg, 4.51 mmol) in EtOH (5 mL) and 3 M HCl (1 mL) in the first step, and then the resulting biphenyldiamine (0.831 mmol) and TEA (0.310 mL, 2.23 mmol) dissolved in DCM (50 mL) was treated with acid chloride **18** (0.742 mmol) in DCM (10 mL), affording a mixture of the monoamides (269 mg, 80%). The monoamide mixture (269 mg, 0.595 mmol) in dioxane (6 mL) was treated with CSA (28.0 mg, 0.119 mmol) at 100 °C for 4 h, yielding a white foam (121 mg, 47%). Treatment of the benzimidazole (113 mg, 0.261 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.260 mL, 0.260 mmol) afforded the title compound as a white powder (112 mg, 91%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.72 (d, $J$ = 8.3 Hz, 1H), 7.63 (s, 1H), 7.55−7.62 (m, 1H), 7.45 (t, $J$ = 8.8 Hz, 1H), 7.40 (d, $J$ = 8.3 Hz, 1H), 7.30 (d, $J$ = 8.3 Hz, 1H), 3.31 (s, 2H), 1.63−1.80 (m, 6H), 1.33−1.57 (m, 4H). HRMS calcd for $C_{22}H_{19}F_4N_3O_2$ (M + H)$^+$ 434.1492, found 434.1494.

**3-[7-Fluoro-5-(2-trifluoromethylphenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (60).** The title compound was prepared according to the general procedure from nitroaniline **41** (300 mg, 1.00 mmol) and Fe powder (279 mg, 5.00 mmol) in EtOH (5 mL) and 3 M HCl (1 mL) in the first step, and then the resulting biphenyldiamine (0.561 mmol) and TEA (0.210 mL, 1.53 mmol) dissolved in DCM (30 mL) was treated with acid chloride **18** (0.510 mmol) in DCM (20 mL), affording a mixture of the monoamides (140 mg, 63%). The monoamide mixture (140 mg, 0.322 mmol) in dioxane (2 mL) was treated with CSA (15.0 mg, 0.064 mmol) at 100 °C for 3 h, yielding a white foam (116 mg, 86%). Treatment of the benzimidazole (116 mg,

0.278 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.280 mL, 0.280 mmol) afforded the title compound as a white powder (110 mg, 87%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.86 (d, $J$ = 7.8 Hz, 1H), 7.74 (t, $J$ = 7.6 Hz, 1H), 7.65 (t, $J$ = 7.6 Hz, 1H), 7.50 (d, $J$ = 7.6 Hz, 1H), 7.26 (s, 1H), 7.03 (d, $J$ = 11.4 Hz, 1H), 3.31 (s, 2H), 1.60−1.81 (m, 6H), 1.29−1.58 (m, 4H). HRMS calcd for $C_{22}H_{19}F_4N_3O$ $(M + H)^+$ 418.1543, found 418.1559.

**3-[7-Chloro-5-(2-trifluoromethylphenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (61).** The title compound was prepared according to the general procedure from nitroaniline **46** (161 mg, 0.508 mmol) and Fe powder (142 mg, 2.54 mmol) in EtOH (3 mL) and 3 M HCl (0.6 mL) in the first step, and then the resulting biphenyldiamine (140 mg, 0.490 mmol) and TEA (0.190 mL, 1.34 mmol) dissolved in DCM (30 mL) was treated with acid chloride **18** (0.445 mmol) in DCM (20 mL), affording a mixture of the monoamides (145 mg, 72%). The monoamide mixture (145 mg, 0.321 mmol) in dioxane (3 mL) was treated with CSA (15.0 mg, 0.064 mmol) at 100 °C for 3 h, yielding a white foam (116 mg, 84%). Treatment of the benzimidazole (116 mg, 0.268 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.270 mL, 0.270 mmol) afforded the title compound as a white powder (103 mg, 82%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 13.52 (br s, 1H), 7.86 (d, $J$ = 7.6 Hz, 1H), 7.75 (t, $J$ = 7.5 Hz, 1H), 7.65 (t, $J$ = 7.6 Hz, 1H), 7.51 (d, $J$ = 7.6 Hz, 1H), 7.35 (s, 1H), 7.25 (s, 1H), 1.63−1.82 (m, 6H), 1.34−1.60 (m, 4H). HRMS calcd for $C_{22}H_{19}ClF_3N_3O$ $(M + H)^+$ 434.1247, found 434.1258.

**2-(1-Oxa-2-aza-spiro[4.5]dec-2-en-3-yl)-6-(2-trifluoromethylphenyl)-3*H*-benzimidazole-4-carbonitrile Hydrochloride (62).** The title compound was prepared according to a modified general procedure from nitroaniline **49** (86.1 mg, 0.280 mmol), $NH_4Cl$ (150 mg, 2.80 mmol), and Fe powder (78.0 mg, 1.40 mmol) in EtOH (3 mL) and $H_2O$ (1 mL) stirred at 80 °C for 1 h, affording the biphenyldiamine (76.0 mg, 98%) in the first step, and then the resulting biphenyldiamine (76.0 mg, 0.274 mmol) and TEA (0.100 mL, 0.747 mmol) dissolved in DCM (25 mL) was treated with acid chloride **18** (0.249 mmol) in DCM (15 mL), affording a mixture of the monoamides (77.7 mg, 71%). The monoamide mixture (77.7 mg, 0.176 mmol) in dioxane (2 mL) was treated with CSA (18.0 mg, 0.077 mmol) at 100 °C for 3 h, yielding a white foam (40.8 mg, 54%). Treatment of the benzimidazole (40.8 mg, 0.096 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.096 mL, 0.096 mmol) afforded the title compound as a white powder (23.7 mg, 54%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.88 (d, $J$ = 7.6 Hz, 1H), 7.74−7.81 (m, 1H), 7.71 (s, 2H), 7.64−7.70 (m, 1H), 7.53 (d, $J$ = 7.6 Hz, 1H), 3.35 (s, 2H), 1.63−1.82 (m, 6H), 1.31−1.59 (m, 4H). HRMS calcd for $C_{23}H_{19}F_3N_4O$ $(M + H)^+$ 425.1589, found 425.1589.

**3-[7-Bromo-5-(2-trifluoromethylphenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (63).** The title compound was prepared according to the general procedure from nitroaniline **47** (98.2 mg, 0.272 mmol) and Fe powder (76.0 mg, 1.36 mmol) in EtOH (2 mL) and 3 M HCl (0.4 mL) in the first step, and then the resulting biphenyldiamine (89.1 mg, 0.269 mmol) and TEA (0.100 mL, 0.735 mmol) dissolved in DCM (15 mL) was treated with acid chloride **18** (0.245 mmol) in DCM (10 mL), affording a mixture of the monoamides (87.9 mg, 72%). The monoamide mixture (87.9 mg, 0.177 mmol) in dioxane (2 mL) was treated with CSA (8.0 mg, 0.035 mmol) at 100 °C for 3 h, yielding a white foam (69.2 mg, 82%). Treatment of the benzimidazole (69.2 mg, 0.145 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.150 mL, 0.150 mmol) afforded the title compound as a white powder (49.5 mg, 66%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.86 (d, $J$ = 7.1 Hz, 1H), 7.75 (t, $J$ = 7.3 Hz, 1H), 7.65 (t, $J$ = 7.7 Hz, 1H), 7.51 (d, $J$ = 7.6 Hz, 1H), 7.41 (s, 1H), 7.39 (s, 1H), 3.33 (s, 2H), 1.62−1.81 (m, 6H), 1.32−1.59 (m, 4H). HRMS calcd for $C_{22}H_{19}BrF_3N_3O$ $(M + H)^+$ 478.0742, found 478.0740.

**3-[5-(2-Fluorophenyl)-7-trifluoromethyl-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (64).** The title compound was prepared according to the general procedure from nitroaniline **42** (268 mg, 0.892 mmol) and Fe powder (249 mg, 4.45 mmol) in EtOH (5 mL) and 3 M HCl (1 mL) in the first step, and then the resulting biphenyldiamine (0.892 mmol) and TEA (0.330 mL, 2.39 mmol) dissolved in DCM (50 mL) was treated with acid chloride **18** (0.796 mmol) in DCM (30 mL), affording a mixture of the monoamides (256 mg, 74%). The monoamide mixture (256 mg, 0.589 mmol) in dioxane (10 mL) was treated with CSA (27.3 mg, 0.118 mmol) at 100 °C for 3 h, yielding a white foam (242 mg, 98%). Treatment of the benzimidazole (242 mg, 0.579 mmol) in EtOAc (3 mL) with 1 M HCl in $Et_2O$ (0.580 mL, 0.580 mmol) afforded the title compound as a white powder (211 mg, 80%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.92 (s, 1H), 7.71 (s, 1H), 7.62−7.70 (m, 1H), 7.44−7.52 (m, 1H), 7.32−7.41 (m, 2H), 3.33 (s, 2H), 1.61−1.83 (m, 6H), 1.35−1.59 (m, 4H). HRMS calcd for $C_{22}H_{19}F_4N_3O$ $(M + H)^+$ 418.1543, found 418.1542.

**3-[5-(2-Trifluoromethoxyphenyl)-7-trifluoromethyl-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (65).** The title compound was prepared according to the general procedure from nitroaniline **44** (326 mg, 0.890 mmol) and Fe powder (249 mg, 4.45 mmol) in EtOH (5 mL) and 3 M HCl (1 mL) in the first step, and then the resulting biphenyldiamine (294 mg, 0.876 mmol) and TEA (0.330 mL, 2.39 mmol) dissolved in DCM (50 mL) was treated with acid chloride **18** (0.796 mmol) in DCM (30 mL), affording a mixture of the monoamides (276 mg, 69%). The monoamide mixture (276 mg, 0.550 mmol) in dioxane (10 mL) was treated with CSA (25.6 mg, 0.110 mmol) at 100 °C for 3 h, yielding a white foam (252 mg, 95%). Treatment of the benzimidazole (252 mg, 0.521 mmol) in EtOAc (3 mL) with 1 M HCl in $Et_2O$ (0.520 mL, 0.520 mmol) afforded the title compound as a white powder (232 mg, 86%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.86 (s, 1H), 7.66−7.73 (m, 1H), 7.64 (s, 1H), 7.50−7.62 (m, 3H), 3.33 (s, 2H), 1.62−1.83 (m, 6H), 1.34−1.61 (m, 4H). HRMS calcd for $C_{23}H_{19}F_6N_3O_2$ $(M + H)^+$ 484.1460, found 484.1458.

**3-[5-(2-Chlorophenyl)-7-trifluoromethyl-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene Hydrochloride (66).** The title compound was prepared according to the general procedure from nitroaniline **45** (291 mg, 0.918 mmol) and Fe powder (256 mg, 4.59 mmol) in EtOH (5 mL) and 3 M HCl (1 mL) in the first step, and then the resulting biphenyldiamine (266 mg, 0.929 mmol) and TEA (0.330 mL, 2.39 mmol) dissolved in DCM (50 mL) was treated with acid chloride **18** (0.796 mmol) in DCM (30 mL), affording a mixture of the monoamides (233 mg, 65%). The monoamide mixture (233 mg, 0.517 mmol) in dioxane (10 mL) was treated with CSA (24.0 mg, 0.103 mmol) at 100 °C for 3 h, yielding a white foam (156 mg, 70%). Treatment of the benzimidazole (156 mg, 0.359 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.360 mL, 0.360 mmol) afforded the title compound as a white powder (115 mg, 68%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 13.52 (br s, 1H), 7.86 (d, $J$ = 7.6 Hz, 1H), 7.75 (t, $J$ = 7.5 Hz, 1H), 7.65 (t, $J$ = 7.6 Hz, 1H), 7.51 (d, $J$ = 7.6 Hz, 1H), 7.35 (s, 1H), 7.25 (s, 1H), 3.33 (s, 2H), 1.63−1.82 (m, 6H), 1.33−1.59 (m, 4H). HRMS calcd for $C_{22}H_{19}ClF_3N_3O$ $(M + H)^+$ 434.1247, found 434.1245.

**3-[2-(1-Oxa-2-aza-spiro[4.5]dec-2-en-3-yl)-6-(2-trifluoromethylphenyl)-3*H*-benzimidazol-4-yl]-propan-1-ol Hydrochloride (67).** The title compound was prepared according to the general procedure from biphenyldiamine **52** (93.2 mg, 0.220 mmol) and TEA (0.0840 mL, 0.600 mmol) dissolved in DCM (20 mL) by slow addition of acid chloride **18** (0.200 mmol) in DCM (10 mL), affording a mixture of the monoamides, which was used as crude material in the next step. The monoamide mixture (0.200 mmol) in dioxane (6 mL) was treated with CSA (9.0 mg, 0.040 mmol) at 100 °C for 3 h, during which time the silyl ether was cleaved concurrently with the cyclization, affording the hydroxypropylbenzimidazole (51.3 mg, 56%). Treatment of the benzimidazole (51.3 mg, 0.112 mmol) in EtOAc (2 mL) with 1 M HCl in $Et_2O$ (0.110 mL, 0.110 mmol) afforded the title compound as a white powder (48.6 mg, 88%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.85 (d, $J$ = 7.6 Hz, 1H), 7.73 (t, $J$ = 7.5 Hz, 1H), 7.63 (t, $J$ = 7.6 Hz, 1H), 7.48 (d, $J$ = 7.3 Hz, 1H), 7.37 (s, 1H), 7.08 (s, 1H), 3.45 (t, $J$ = 6.6 Hz, 2H), 3.33 (s, 2H), 3.01 (t, $J$ = 7.5 Hz, 2H), 1.79−1.90 (m, 2H), 1.61−1.79 (m, 6H), 1.31−1.57 (m, 4H).

HRMS calcd for $C_{25}H_{26}F_3N_3O_2$ $(M + H)^+$ 458.2055, found 458.2055.

**3-[5-(2-Trifluoromethylphenyl)-1*H*-benzimidazol-2-yl]-1,8-dioxa-2-aza-spiro[4.5]dec-2-ene (68).** The title compound was prepared according to the general procedure from biphenyldiamine **7** (131 mg, 0.520 mmol) and TEA (0.19 mL, 1.39 mmol) dissolved in DCM (50 mL) by slow addition of acid chloride **19** (0.464 mmol) in DCM (15 mL), affording a mixture of the monoamides (133 mg, 68%). The monoamide mixture (133 mg, 0.317 mmol) in dioxane (6 mL) was treated with CSA (15.0 mg, 0.063 mmol) at 100 °C for 4 h, yielding a white foam (27.6 mg, 22%). $^1$H NMR (400 MHz, DMSO-$d_6$ + TFA-$d_1$) $\delta$: 7.86 (d, $J$ = 7.6 Hz, 1H), 7.71−7.78 (m, 1H), 7.68 (d, $J$ = 8.3 Hz, 1H), 7.60−7.67 (m, 1H), 7.55 (s, 1H), 7.48 (d, $J$ = 7.3 Hz, 1H), 7.26 (d, $J$ = 9.1 Hz, 1H), 3.75−3.87 (m, 2H), 3.54−3.68 (m, 2H), 3.42 (s, 2H), 1.79−1.94 (m, 4H). HRMS calcd for $C_{22}H_{18}F_5N_3O$ $(M + H)^+$ 402.1429, found 402.1432.

**8,8-Difluoro-3-[5-(2-trifluoromethylphenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro[4.5]dec-2-ene (69).** The title compound was prepared according to the general procedure from biphenyldiamine **7** (97.5 mg, 0.386 mmol) and TEA (0.14 mL, 1.04 mmol) dissolved in DCM (50 mL) by slow addition of acid chloride **20** (0.345 mmol) in DCM (15 mL), affording a mixture of the monoamides (103 mg, 66%). The monoamide mixture (103 mg, 0.228 mmol) in dioxane (1 mL) was treated with CSA (11.0 mg, 0.046 mmol) at 100 °C for 4 h, yielding a white foam (53.5 mg, 54%). $^1$H NMR (400 MHz, DMSO-$d_6$ + TFA-$d_1$) $\delta$: 7.85 (d, $J$ = 7.8 Hz, 1H), 7.71−7.78 (m, 1H), 7.69 (d, $J$ = 8.3 Hz, 1H), 7.59−7.67 (m, 1H), 7.56 (s, 1H), 7.48 (d, $J$ = 7.6 Hz, 1H), 7.27 (d, $J$ = 8.3 Hz, 1H), 3.45 (s, 2H), 1.85−2.23 (m, 8H). HRMS calcd for $C_{22}H_{18}F_5N_3O$ $(M + H)^+$ 436.1448, found 436.1462.

**Preparation of the Spiro-isoxazoline Benzimidazoles 70, 71: General Procedure.** The biphenylnitroaniline (1 equiv) in dry THF (50 mL) was cooled to 0 °C in an ice bath, and then NaH (3 equiv, 60% dispersion in oil) was added in small portions. Spiro-isoxazoline acid chloride **19** (1.1 equiv) in dry THF (20 mL) was added dropwise to the reaction mixture over 10 min, and then the mixture was stirred for 30 min at 0 °C, warmed to rt, and stirred for an additional 16 h. The mixture was quenched with satd aq $NH_4Cl$ solution and extracted three times with EtOAc. The combined organic extracts were dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure, and then the residue was purified by column chromatography to afford the nitroamide. The nitroamide was dissolved in a mixture of AcOH and MeOH (4:1) and treated with Fe powder (5 equiv). The mixture was heated to 100 °C for 4 h, and then the solvent was removed under reduced pressure. The residue was dissolved in EtOAc and washed with satd aq $NaHCO_3$ solution. The aqueous layer was extracted twice with EtOAc, and then the combined organic extracts were dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure, and then the crude material was chromatographed on an 80 g $SiO_2$ prepacked column eluting with 0:1 to 2:3 EtOAc/hexanes, affording the benzimidazole. The benzimidazole was dissolved in EtOH, and then 5 M HCl in IPA (1 equiv) was added. The reaction was stirred at rt for 30 min, and then the solvent was removed under high vacuum to afford the benzimidazole hydrochloride salt.

**3-[7-Trifluoromethyl-5-(2-trifluoromethylphenyl)-1*H*-benzimidazol-2-yl]-1,8-dioxa-2-aza-spiro[4.5]dec-2-ene (70).** The title compound was prepared according to the general procedure from nitroaniline **43** (200 mg, 0.571 mmol) and NaH (68.5 mg of 60% mineral oil dispersion, 1.71 mmol) in THF (10 mL) at 0 °C and acid chloride **19** (0.628 mmol) in THF (5 mL), affording the nitroamide (178 mg, 60%). The nitroamide (178 mg, 0.344 mmol) in a mixture of AcOH (8 mL) and MeOH (2 mL) was treated with Fe powder (96.1 mg, 1.72 mmol) at 100 °C for 4 h, yielding a colorless glass (144 mg, 89%). Treatment of the benzimidazole (144 mg, 0.307 mmol)) in EtOH (2 mL) with 5 M HCl in IPA (61.4 $\mu$L, 0.307 mmol) afforded the title compound as a colorless foam (107 mg, 69%). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 7.86 (d, $J$ = 7.6 Hz, 1H), 7.83 (s, 1H), 7.69−7.76 (m, 1H), 7.59−7.67 (m, 2H), 7.49 (d, $J$ = 7.6 Hz, 1H), 3.88−3.97 (m, 2H), 3.72−3.81 (m, 2H), 3.47 (s, 2H), 1.94−2.00 (m, 4H). HRMS calcd for $C_{22}H_{17}F_6N_3O_2$ $(M + H)^+$ 470.1303, found 470.1301.

**3-[7-Chloro-5-(2-trifluoromethyl-phenyl)-1*H*-benzimidazol-2-yl]-1,8-dioxa-2-aza-spiro[4.5]dec-2-ene (71).** The title compound was prepared according to the general procedure from nitroaniline **46** (174 mg, 0.549 mmol) and NaH (65.8 mg of 60% mineral oil dispersion, 1.65 mmol) in THF (10 mL) at 0 °C and acid chloride **19** (0.604 mmol) in THF (5 mL), affording the nitroamide (126 mg, 48%). The nitroamide (126 mg, 0.260 mmol) in a mixture of AcOH (8 mL) and MeOH (2 mL) was treated with Fe powder (72.7 mg, 1.30 mmol) at 100 °C for 4 h, yielding a colorless glass (103 mg, 91%). Treatment of the benzimidazole (103 mg, 0.235 mmol)) in EtOH (2 mL) with 5 M HCl in IPA (47.0 $\mu$L, 0.235 mmol) afforded the title compound as a colorless foam (50.0 mg, 45%). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 7.84 (d, $J$ = 7.6 Hz, 1H), 7.71 (t, $J$ = 7.3 Hz, 1H), 7.63 (t, $J$ = 7.6 Hz, 1H), 7.57 (br s, 1H), 7.47 (d, $J$ = 6.8 Hz, 2H), 3.87−3.97 (m, 2H), 3.73−3.82 (m, 2H), 3.45 (s, 2H), 1.95−2.01 (m, 4H). HRMS calcd for $C_{21}H_{17}ClF_3N_3O_2$ $(M + H)^+$ 436.1040, found 436.1037.

**3-(5-Bromo-1*H*-benzo[*d*]imidazol-2-yl)-1-oxa-2-azaspiro[4.5]dec-2-ene (73).** 4-Bromobenzene-1,2-diamine (**72**) (377 mg, 2.02 mmol) was dissolved in DCM (100 mL) then TEA (0.65 mL, 4.65 mmol) was added. A DCM (50 mL) solution of acid chloride **18** (1.55 mmol) was added dropwise over 10 min, and then the reaction was stirred at rt for 1 h. The solvent was removed under reduced pressure, and then the residue was purified by column chromatography using an 80 g $SiO_2$ prepacked column eluting with 0:1−2:3 EtOAc/hexanes to afford a mixture of the two monoamides (460 mg, 84%). The monoamide mixture (460 mg, 1.31 mmol) was dissolved in anhydrous dioxane (10 mL), and then CSA (61.0 mg, 0.261 mmol) was added and the reaction was stirred at 100 °C for 3 h. The reaction was cooled to rt, the acid was quenched with TEA (0.1 mL), and then the reaction mixture was purified by chromatography on a 40 g $SiO_2$ prepacked column eluting with 0:1−2:3 EtOAc/hexanes to afford the title compound (299 mg, 68%). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 7.74 (s, 1H), 7.51 (d, $J$ = 8.6 Hz, 1H), 7.40 (dd, $J$ = 8.6, 1.8 Hz, 1H), 3.25 (s, 2H), 1.69−1.88 (m, 6H), 1.43−1.63 (m, 4H).

**3-[5-(2-Chlorophenyl)-1*H*-benzimidazol-2-yl]-1-oxa-2-aza-spiro-[4.5]dec-2-ene Hydrochloride (74).** The title compound was prepared from **73** (148 mg, 0.442 mmol), 2-chlorophenylboronic acid (**27**) (90.0 mg, 0.575 mmol), and (dppf)$PdCl_2$·DCM (18.0 mg, 0.0220 mmol) in DME (2 mL) and 2 M aq $Na_2CO_3$ (0.500 mL, 1.00 mmol) according to the general Suzuki procedure, yielding a white solid (75.7 mg, 47%). Treatment of the benzimidazole (75.7 mg, 0.207 mmol) in EtOAc (1 mL) with 1 M HCl in $Et_2O$ (0.200 mL, 0.200 mmol) afforded the title compound as a white powder (74.3 mg, 89%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.71 (d, $J$ = 8.6 Hz, 1H), 7.66 (s, 1H), 7.58−7.62 (m, 1H), 7.41−7.51 (m, 3H), 7.39 (dd, $J$ = 8.5, 1.6 Hz, 1H), 3.31 (s, 2H), 1.63−1.80 (m, 6H), 1.36−1.55 (m, 4H). HRMS calcd for $C_{21}H_{20}ClN_3O$ $(M + H)^+$ 366.1373, found 366.1372.

**2-{2-[2-(1-Oxa-2-aza-spiro[4.5]dec-2-en-3-yl)-1*H*-benzimidazol-5-yl]-phenyl}-propan-2-ol (75).** The title compound was prepared from **73** (150 mg, 0.448 mmol), 3,3-dimethyl-3*H*-benzo[*c*][1,2]oxaborol-1-ol[12] (DMBB) (145 mg, 0.898 mmol), and (dppf)$PdCl_2$·DCM (37.0 mg, 0.0450 mmol) in DME (2 mL) and 2 M aq $Na_2CO_3$ (0.75 mL, 1.50 mmol) according to the general Suzuki procedure, yielding a white solid (16.4 mg, 9.4%). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$: 7.82 (dd, $J$ = 8.1, 1.3 Hz, 1H), 7.53−7.68 (m, 1H), 7.47 (br s, 1H), 7.35 (td, $J$ = 7.6, 1.6 Hz, 1H), 7.19 − 7.24 (m, 2H), 7.05 (dd, $J$ = 7.6, 1.3 Hz, 1H), 3.28 (s, 2H), 1.69−1.90 (m, 6H), 1.46−1.65 (m, 4H), 1.33 (s, 6H). HRMS calcd for $C_{24}H_{27}N_3O_2$ $(M + H)^+$ 390.2182, found 390.2174.

**Biological Assays. In Vitro Canine TRPM8 Functional Assay.** TRPM8 functional activity was initially determined by measuring changes in intracellular calcium concentration using a $Ca^{2+}$-

sensitive fluorescent dye. The changes in fluorescent signal were monitored by a fluorescence plate reader, either FLIPR manufactured by Molecular Devices or FDSS manufactured by Hamamatsu. Increases in intracellular $Ca^{2+}$ concentration were readily detected upon activation with icilin.

At 24 h prior to the assay, HEK293 cells stably expressing canine TRPM8 were seeded in culture medium in black wall, clear-base poly-D-lysine coated 384-well plates (BD Biosciences, NJ, USA) and grown overnight in 5% $CO_2$ at 37 °C. On assay day, the growth media was removed and cells were loaded with Calcium 3 Dye (Molecular Devices) for 35 min at 37 °C under 5% $CO_2$ and then for 25 min at rt. Subsequently, cells were tested for agonist-induced increases in intracellular $Ca^{2+}$ levels using FLIPR or FDSS. Cells were then exposed to test compound (at varying concentrations), and intracellular $Ca^{2+}$ was measured for 5 min prior to the addition of icilin to all wells to achieve a final concentration that produces approximately an 80% maximal response. $EC_{50}$ or $IC_{50}$ values were determined from eight-point concentration−response studies. Curves were generated using the average of quadruplicate wells for each data point.

**In Vitro Canine TRPM8 Electrophysiology.** HEK293 cells stably expressing cTRPM8 were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 400 μg/mL G418. Cells were plated on glass coverslips, maintained at 37 °C and in 5% $CO_2$, and used 1−2 days after plating.

Recordings were performed using the conventional whole-cell patch clamp technique. Currents were continuously sampled (at 100 Hz)/recorded using Digidata 1322A/pClamp 9.0 and amplified and filtered (at 2 kHz) using Axopatch 200B. The holding potential was −60 mV. The extracellular solution contained (in mM): NaCl (132), KCl (5.4), $MgCl_2$ (0.8), EGTA (1), HEPES (10), and glucose (10), pH = 7.4. The intracellular solution used to fill recording pipettes contained (in mM): CsCl (145), EGTA (5), HEPES (10), and glucose (5), pH = 7.4.

The temperature of the solution perfusing the cell was cooled to 10 °C from 22 °C to activate the channel using an in-line cooler. After steady-state current activation was achieved, one or several concentrations (sequentially applied with increasing concentration) of test compound were applied to the cell at 10 °C. This was followed by the application of a saturating concentration of a reference antagonist (also at 10 °C) to establish a baseline current from which the other currents were subtracted.

Percent inhibition at a given concentration of test compound was calculated as the ratio between the amplitudes of the current in the presence and absence of that concentration of test compound. The concentration−response data were fitted to a logistic function as follows: $R = 100/(1 + c/\mathrm{IC50})p$, where, $R$ is the percentage response, $p$ is the Hill coefficient, and $c$ is the concentration of test compound.

**In Vivo Assays. Icilin-Induced "Wet-Dog" Shaking in Rats.** To assess the ability of a TRPM8 antagonist to prevent icilin-induced shaking behavior in rats, test compounds (3−30 mg/kg, po, in 20% HPβCD; $n$ = 6−7/group) were administered 1−2 h prior to icilin challenge (3 mg/kg ip in 10% Solutol/water). Spontaneous WDS were counted over a 10 min period, 10−20 min posticilin. Inhibition of the spontaneous WDS behavior relative to vehicle pretreatment is expressed as percent inhibition, calculated as follows: % Inhibition = [1 − (treatment WDS count/vehicle WDS count)] × 100.

**Chronic Constriction Injury (CCI) Acetone Test.** In male Sprague−Dawley rats (Harlan Laboratories, Indianapolis, IN; 175−225 g at surgery), four loose ligatures of 4−0 chromic gut were surgically placed around the left sciatic nerve under inhalation anesthesia.[15] Two weeks later, the rats were placed individually in elevated observation chambers having wire mesh floors. A series of five applications of acetone (0.10 mL/application) was gently sprayed onto the bottom of the paw using a multidose syringe device in a modification of a previously described method.[23] A positive response took the form of an abrupt or protracted withdrawal or lifting of the paw. Rats were acclimated to this test procedure over the course of several practices sessions. Only rats exhibiting a 100% positive response rate (5 of 5) at the time of the predose baseline were included in the study. The inhibition of positive responses following administration of test compound ($n$ = 7−13 rats/treatment) was taken as a percentage of the number of positive responses prior to test compound treatment according to the following formula: % iInhibition = [1 − (treatment responses/baseline responses)] × 100. Time course data were analyzed by ANOVA, followed by a Bonferroni posthoc test. $ED_{50}$ values and associated statistics were calculated using PharmTools Plus software (The McCary Group, Schnecksville, PA).

**Cold Pressor Test.** Fasted, male, Sprague−Dawley rats (300−450 g) were orally administered **5** (0.3, 1, 3, 10, or 30 mg/kg) or vehicle (20% HPβCD) 120 min prior to cold pressor testing. Then 75 min following oral dose administration, rats were anesthetized with 65 mg/kg ip sodium pentobarbital, followed by 10 mg/kg/h supplemental doses, with continuous intravenous infusion (jugular cannulation) until the end of the study. A surgically placed carotid artery catheter was connected to a pressure transducer to continuously record blood pressure. Cold stimulation of the fore paws was accomplished by placing the paws in ice water for 3−5 min, followed by active rewarming with rt water. The percent change in mean arterial pressure in response to this cold stimulus was calculated for vehicle and test compound pretreatments. Percent inhibition attributed to treatment with test compound was then determined using the following formula:

$$\%\text{inhibition} = [1 - (\text{cold evoked}\%\text{change in BP post} - \text{test compound})/(\text{cold evoked}\%\text{change in BP post} - \text{vehicle})] \times 100$$

Blood sampling was performed at the end of the experiment to assess compound exposure levels.

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