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Discovery and Optimization of 1-(4-(Pyridin-2-yl)benzyl)-imidazolidine-2,4-dione Derivatives As a Novel Class of Selective Cannabinoid CB2 Receptor Agonists

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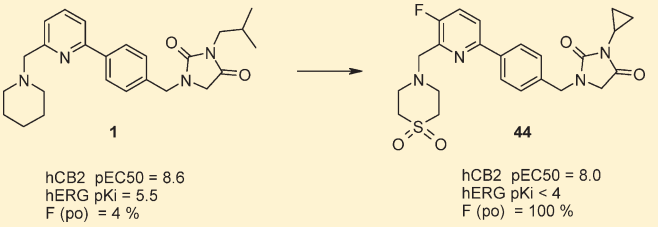
ABSTRACT: Here, we report the identification and optimization of 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione derivatives as a novel chemotype with selective cannabinoid CB2 receptor agonist activity. **1** is a potent and selective cannabinoid CB2 receptor agonist (hCB2 pEC_{50} = 8.6). The compound was found to be metabolically unstable, which resulted in low oral bioavailability in rat (F_{po} = 4%) and possessed off-target activity at the hERG ion channel (pK_i = 5.5). Systematic modification of physicochemical properties, such as lipophilicity and basicity, was used to optimize the pharmacokinetic profile and hERG affinity of this novel class of cannabinoid CB2 receptor agonists. This led to the identification of **44** as a potent, selective, and orally bioavailable cannabinoid CB2 receptor agonist (hCB2 pEC_{50} = 8.0; hERG pK_i < 4; F_{po} = 100%), which was active in a rat spinal nerve ligation model of neuropathic pain.



INTRODUCTION

Neuropathic pain, defined as chronic pain caused by injury, disease, or dysfunction of the nervous system, is present in ~1% of the population; the largest patient populations include those with painful diabetic peripheral neuropathy and those with neuralgia that persists after an attack of herpes zoster (postherpetic neuralgia). It is characterized by a complex combination of symptoms, including spontaneous pain that can occur in the absence of tissue damage. Patients suffering from neuropathic pain also have increased sensitivity both to stimuli normally perceived as painful (hyperalgesia) as well as to stimuli that do not normally provoke pain (allodynia). These symptoms are often refractory to conventional analgesic therapies, with most patients achieving incomplete relief of their symptoms. Currently, antidepressants, anticonvulsants, and opioids remain first-line treatment, with pregabalin as the gold standard. All of these drugs have significant side effects that are dose limiting. In addition, efficacy is a considerable problem in the neuropathic pain market, with current treatments showing a maximum of 50% reduction in overall pain scores from baseline. Consequently, there remains an unmet medical need for agents that have higher efficacy/responder rate and with reduced side-effects compared with currently used drugs.[1,2]

Marijuana has been used in the treatment of various kinds of ailments for centuries.[3] Emerging clinical evidence, as well as anecdotal reports from patients self-medicating with cannabis, suggest that cannabinoid receptor agonists may have a role in treating pain.[4,5] The antinociceptive properties of Δ^9-tetrahydrocannabinol, the main psychoactive ingredient in cannabis, have been well established in preclinical models, but its therapeutic effect in man is limited by its undesirable psychotropic activities and abuse potential.[3,4] Δ^9-Tetrahydrocannabinol acts on two G-protein coupled receptors, namely the cannabinoid CB1 and CB2 receptors.[6] The cannabinoid CB1 receptor is abundantly expressed in the central nervous system (CNS) and is responsible for the psychotropic side effects.[3,6,7] The cannabinoid CB2 receptor is mainly found in cells of the immune system and is upregulated in the CNS under pathological conditions.[8,9] For example, Anand et al. describe the expression of cannabinoid CB2 receptor on human sensory neurons, which increased after trauma. They demonstrated that the cannabinoid CB2 receptor is colocalized with TRPV1 and suggest that CB2 receptor agonists reduce Ca^{2+} influx by blockade of TRPV1 receptor activation (via depletion of cAMP).[10]

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The focus of drug discovery efforts have recently turned to the cannabinoid CB2 receptor, because selective activation of this receptor has been shown to elicit analgesic effects in animals without the CNS-mediated side effects typical of a nonselective CB receptor agonist such as THC.[11−13] It has been shown that CB2 receptor expression on monocytes, microglial, and astrocytic cells is of crucial importance to establish neuropathic pain in a sciatic nerve injury model.[14] In CB2 receptor KO mice, allodynia and hyperalgesia were not restricted to the injured limb, but mice also developed a contralateral mirror image pain which was also associated with increased microglial and astrocytic activation; both the behavioral and histological changes were attenuated in CB2 receptor overexpressing mice. The neuropathic pain phenotype of CB2 receptor KO mice was also manifested in irradiated wild-type mice reconstituted with bone marrow cells from CB2 receptor KO mice, thereby demonstrating the implication of the CB2 receptor on immune cells in the development of neuropathic pain in this model. Enhanced IFN-γ signaling was present in CB2 receptor KO mice, but the double KO mice (of CB2 and IFN-γ) did not show any longer the enhanced manifestations of neuropathic pain observed in the CB2 receptor KO mice.[15]

Besides evidence from genetically engineered animals, several selective CB2 receptor agonists from different chemotypes have also been reported to be effective in neuropathic pain models in rodents (see Thakur et al. for a review).[16,17] For example, HU-308,[18] AM1241,[19] GW842166X,[20] and JWH-133[21] belong to the first classes of selective cannabinoid CB2 receptor agonists that have been used to study the action of the cannabinoid CB2 receptor in preclinical models of pain and other diseases, e.g. osteoporosis, multiple sclerosis, melanoma, and neurodegenerative diseases (ALS, Alzheimer's, and Huntington's diseases).[8,9]

Here, we report the identification and optimization of 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione derivatives as a novel chemotype with selective cannabinoid CB2 receptor agonist activity. The initial hit **1** was found to be metabolically unstable, which resulted in low oral bioavailability and possessed off-target activity at the hERG ion channel. In this paper, we describe the optimization of pharmacokinetic properties and hERG affinity of this novel class of cannabinoid CB2 receptor agonists by systematically modulating lipophilicity and basicity. This led to the identification of **44** as a potent, selective, and orally bioavailable cannabinoid CB2 receptor agonist, which was active in a rat spinal nerve ligation model of neuropathic pain.

RESULTS AND DISCUSSION

A focused target family library screen, with proprietary compounds, followed by a small optimization campaign resulted in the discovery of **1**, which is a low molecular weight compound (420 Da) with moderate lipophilicity (Log D = 2.8) and high basicity (pK_a = 8.9). **1** belongs to a novel chemical class of 1-(4-(pyridin-2-yl)benzyl)-imidazolidine-2,4-dione derivatives. **1** has a pEC_{50} = 8.6 for the cannabinoid CB2 receptor and was more than 1000-fold selective over the cannabinoid CB1 receptor in a functional assay using the inhibition of forskolin-induced cAMP formation as a read out in CHO cells stably overexpressing the human recombinant cannabinoid CB2 or CB1 receptors. Because of its physicochemical properties, it was anticipated that the compound may also have an affinity for the hERG ion channel. Compounds that display a high affinity for hERG ion channels are considered to have an increased probability of cardiotoxicity.[22] To evaluate compounds for their inhibition of hERG channels, a displacement assay was performed utilizing hERG channels stably expressed in HEK293 cells and radioligand [^{3}H]-dofetilide, which is a class III antiarrhythmic drug with high affinity for the hERG channel.[22] **1** was found to displace [^{3}H]-dofetilide with a pK_i of 5.5.

Table 1. Comparison of Rat Plasma PK for Compounds 1 and 44 [a]

	1	44
CL (mL/min/kg)	100 ± 12	8.1 ± 2.6
V_{ss} (L/kg)	5.4 ± 1	0.94 ± 0.08
$T_{1/2}$ (h)	0.8	1.7 ± 0.7
F (%)	4 ± 2	100 ± 19
oral C_{max} (μM)	0.02	3.8 ± 0.9
plasma f_u	0.25	0.44
oral $C_{max,u}$ (μM)	0.006	1.7

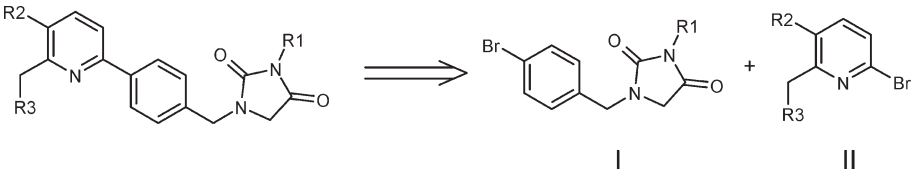
[a] Compounds dosed at 1 mg/kg iv and 5 mg/kg po to fed male Wistar rats (n = 4 per route). Both compounds were dosed orally in 0.5% (w/v) gelatin in 5% (w/v) mannitol.

1 has good Caco-2 permeability (210 nm/s), which was used as an in vitro model of intestinal absorption. Low metabolic stability was observed in human and rat liver microsomes ($t_{1/2}$ = 24 and 21 min, CL_{int} = 58 and 64 μL/min/mg, respectively), which were used as in vitro models to estimated in vivo hepatic clearance.

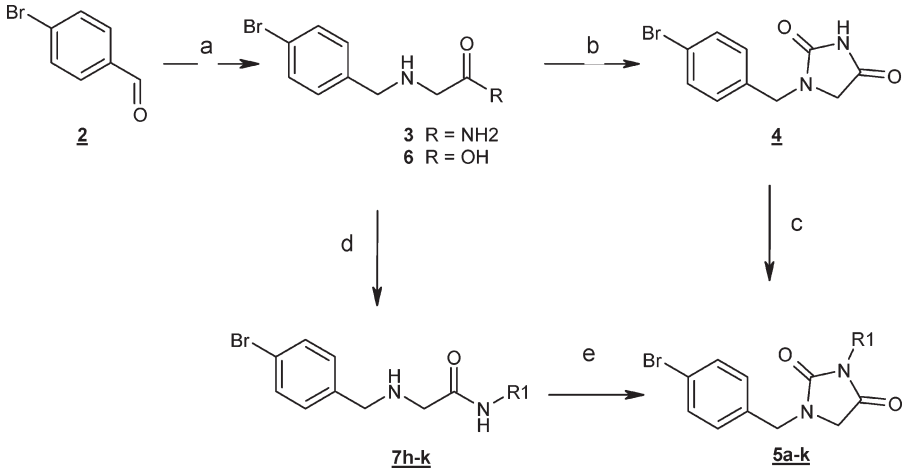
Rat pharmacokinetic studies were performed to test whether sufficient exposure levels could be reached in the blood after oral dosing to modulate cannabinoid CB2 receptor activity in vivo. It was found that **1** had low oral bioavailability (4%) and a C_{max} of 0.024 μM after oral dosing at 5 mg/kg in 0.5% gelatin in 5% mannitol (Table 1). These low exposure levels after oral dosing could be explained by high first pass extraction due to high clearance (100 mL/min/kg).

We set out to systematically modulate the physicochemical properties of the 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione series to improve the ADME properties. A synthetic route was designed which would allow the rapid and simultaneous optimization at three variation points (i.e., R1, R2, and R3) in the molecule. Our approach for the synthesis of the 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione derivatives was first to prepare strategic building blocks I and II (Scheme 1). Depending on the type of substituent at R_1, building block I can be prepared via two methods, both starting from the commercially available 4-bromobenzaldehyde (Scheme 2). In method 1, 4-bromobenzaldehyde was converted via a reductive amination with glycinamide hydrochloride to 2-(4-bromobenzylamino)acetamide **3**. Under basic conditions and in the presence of CDI, the resulting intermediate was converted to 1-(4-bromobenzyl)-imidazolidine-2,4-dione **4**. The R_1-group was introduced by alkylation of **4** with an appropriate alkylhalide in the presence of K_2CO_3, which gave **5a**−**g** in good yields. In case R_1 = cyclopropyl, cyclobutyl, isopropyl, or 2,2,2-trifluoro-ethyl, the substituent was introduced according to method 2 because the corresponding alkyl halides did not react properly. Glycine hydrochloride was used for the reductive amination with 4-bromobenzaldehyde to form 2-(4-bromobenzylamino)acetic acid **6**. Amide coupling using PyBob and the appropriate amine gave **7h**−**k**. The corresponding imidazolidine-2,4-dione derivatives **5h**−**k** were obtained by a ring closure reaction of **7h**−**k**, as previously described.

Scheme 1. Synthetic Strategy for Synthesis of 1-(4-(Pyridin-2-yl)benzyl)imidazolidine-2,4-dione Derivatives

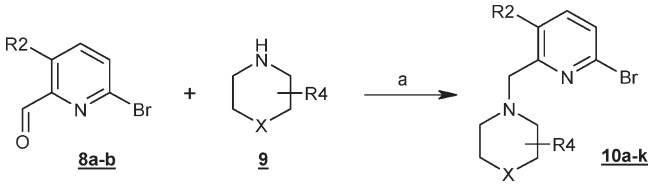


Scheme 2. Synthesis of the 1-(4-Bromobenzyl)-imidazolidine-2,4-dione (Building Block I)[a]



[a] (a) Glycinamide or glycine hydrochloride, CH_3OH/H_2O, NaOH, $NaBH_4$, 0 °C or rt; (b) CDI, DMAP, CH_3CN, 60 °C; (c) alkylhalide, K_2CO_3, 50 °C; (d) alkylamine, PyBOP, Et_3N, CH_2Cl_2, rt; (e) CDI,DMAP, CH_3CN, 60 °C

Scheme 3. Synthesis of 2-Bromopyridine (Building Block II)[a]



[a] (a) HOAc, $NaBH(OAc)_3$,CH_2Cl_2, 10 °C.

Building blocks II **10a**−**k** were prepared via reductive amination of 6-bromopicolinaldehyde **8a** or 6-bromo-3-fluoropicolinaldehyde **8b** with the appropriate amine (Scheme 3). For preparation of the cyclohexyl derivative **15** (Scheme 4), 2,6-dibromopyridine **11** was used as a starting point. After bromo/lithium exchange of **11**, it was reacted with cyclohexanecarbonitrile to afford (6-bromopyridin-2-yl)(cyclohexyl)methanone **13**. Compound **13** was converted into the 4-methylbenzenesulfonohydrazide **14**, which was subsequently reduced to **15**.

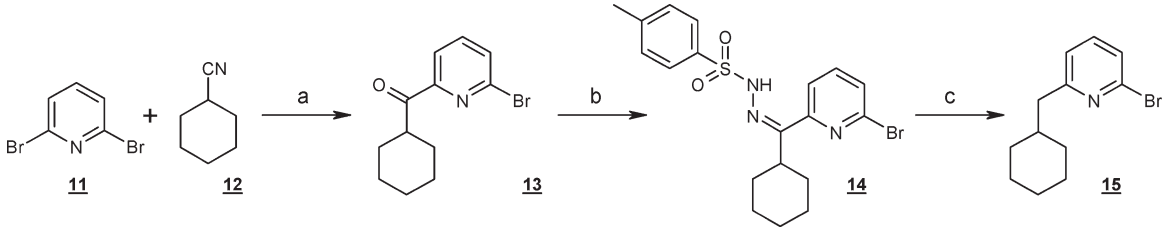
Final compounds were prepared by a Suzuki coupling of the two building blocks **10** and **5** using tetrakis(triphenylphosphine)palladium-(0) as a catalyst (Scheme 5). It should be noted that building blocks **5a**−**k** were first converted to the corresponding boronic esters and used directly in the Suzuki coupling without purification because the boronic esters were unstable during SiO_2 column chromatography. Final products **1**,**18**−**44** were obtained in 3−80% yield.

First, the effect of reducing lipophilicity was investigated by introducing smaller alkyl groups at R_1. Replacing the isobutyl group by a propyl **18**, ethyl **19**, or methyl **20** group decreased log *D* from 2.8 to 1.3 and led to an improved metabolic stability, i.e. the half-life time increased from 22 to 90 min in rat liver microsomes. This was a general trend because the metabolic half-life time of other branched alkyl **21** or cycloalkyls **22**−**24** analogues correlated also with the lipophilicity (Figure 1a). Introducing fluorine at the alkyl chain to block oxidation increased the metabolic stability as observed with compounds **25** and **26**, whereas fluorination of the scaffold at the 5-pyridyl position **27**−**33** was detrimental to metabolic stability. The most lipophilic compound **40** (log *D* = 4.7) was also the most labile compound with a half-life time of 3 min (CL_{int} > 270 μL/min/mg).

The potency of the compounds at the cannabinoid CB2 receptor was also dependent on the lipophilicity of the alkyl chain (Table 2; Figure 1b). There was an inverse correlation with the metabolic stability. Increasing the lipophilicity from 1.3 in compound **20** to 2.2 increased the potency by 50-fold, giving a pEC_{50} = 8.9 for **18**. Replacement of the hydrogen at R_2 by a fluorine increased potency in most cases (e.g., **27** vs **19**; **30** vs **22**; **33** vs **25**). The most potent compound identified in this series was **28** with a pEC_{50} = 9.1. Interestingly, there was no apparent correlation of lipophicity with cannabinoid CB1 receptor activity. All compounds are weak partial cannabinoid CB1 receptor agonists (Table 2). However, lipophilicity did correlate with affinity for the hERG channel. Molecules with smaller alkyl groups at R_1 than an isobutyl group did have lower affinity for hERG compared to **1** (Figure 2a).

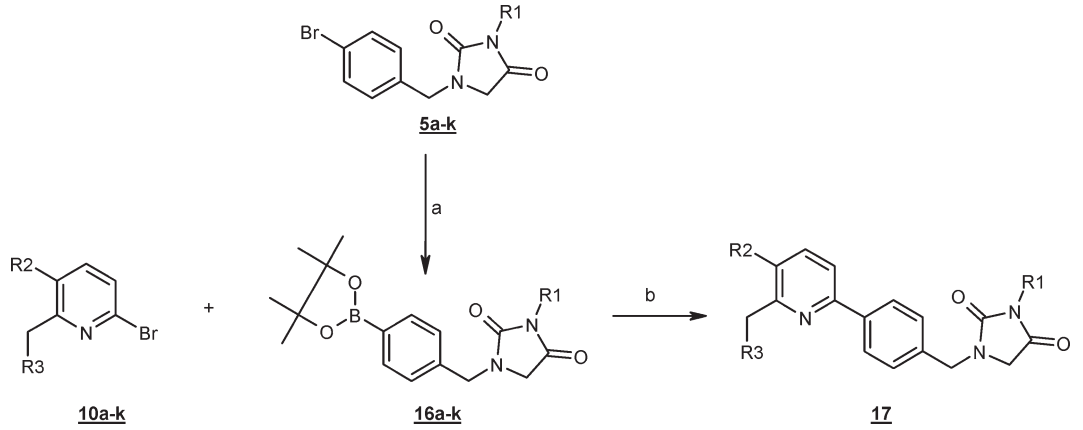
Second, we investigated the influence of the basicity of the amine substituent at the R_3 position by introducing different electron modulating groups in the piperidine ring **19**−**28**, while isobutyl was chosen as the R_1 substituent. The cannabinoid CB2 receptor potency was not affected by the level of basicity (Table 2). Both very basic compounds (i.e., **1** with a pK_a = 8.9) and weakly basic compounds (i.e., **42** with a pK_a = 3.0) were very

Scheme 4. Synthesis of 2-Bromo-6-cyclohexylmethyl-pyridine[a]



[a] (a) BuLi, hexane/THF, −78 °C, H_2SO_4; (b) 4-methylbenzenesulfonyl hydrazide, EtOH, 100 °C; (c) DiBAlH, CH_2Cl_2, rt.

Scheme 5. Synthesis of 1-(4-(Pyridin-2-yl)benzyl)imidazolidine-2,4-dione Derivatives[a]



[a] (a) Bis(pinacolato)diboron, KOAc, Pd(dppf)Cl_2, DMF, 75 °C; (b) K_2CO_3, Pd(PPh_3)$_4$, toluene/EtOH, 75 °C.

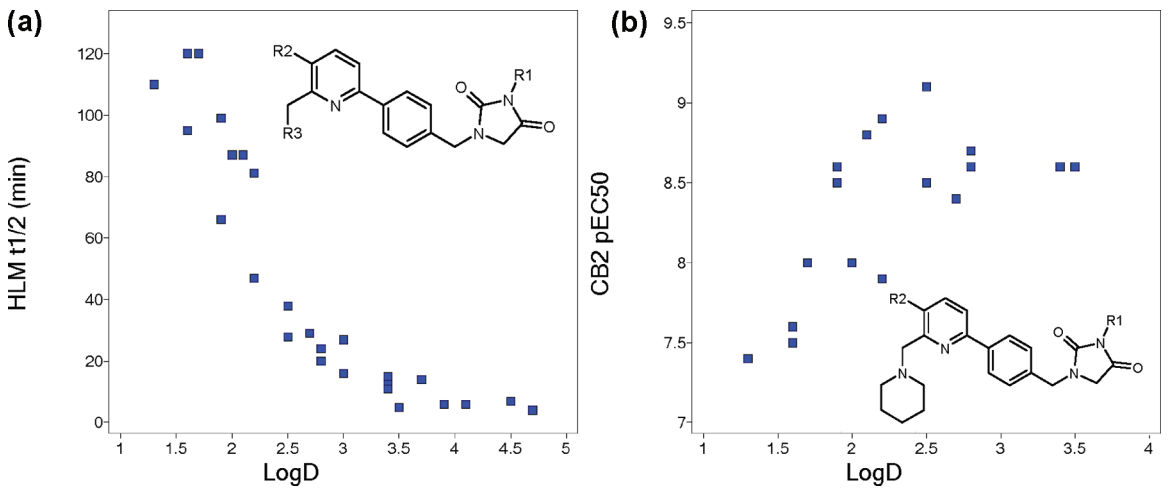


Figure 1. Correlation of human liver microsomal half-life time (a) and potency on human cannabinoid CB2 receptors (b) with lipophilicity of 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione derivatives.

potent cannabinoid CB2 receptor agonists (pEC_{50} = 8.6 for both). Moreover, **43**, in which the nitrogen was replaced by a carbon, did not lose potency (pEC_{50} = 7.7). This indicates that the amine is not essential for the interaction with the cannabinoid CB2 receptor. On the other hand, the hERG ion channel is well-known for its preference of ligands with a basic amine group.[23] This provided an opportunity to separate the biological activity of this chemical series at the cannabinoid CB2 receptor from the hERG channel. Indeed, we have found that lowering the basicity of the amine at R_3 reduced the affinity of the compounds at the hERG channel (Figure 2b) while cannabinoid CB2 receptor potency was maintained (Table 2). For example, **42** (pK_a = 3) had only marginal affinity (27% @ 0.1 mM) for the hERG channel, while its potency at the cannabinoid CB2 receptor was high (pEC_{50} = 8.6). Unfortunately, the metabolic stability of these compounds was very low, which could be attributed to the high lipophilicity (Table 2).

The next step was to combine the substituents at the R_1, R_2, and R_3 position. To avoid the elaborate synthesis of all possible combinations, we have generated COMFA models for CB2

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Table 2. Structure−Property Relationships of 1-(4-(Pyridin-2-yl)benzyl)imidazolidine-2,4-dione Derivatives

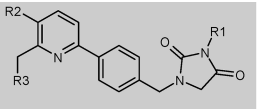
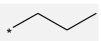
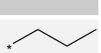
No				Synthesis			Phys Chem			CB2	CB1	hERG	HLM	RLM
	R1	R2	R3	Building blocks		yield %	MW Da	LogD	pK_a	pEC_{50}± CI	% ± SD @10^{-5} M	% ± SD @ 10^{-4} M	$t_{1/2}$ min	$t_{1/2}$ min
1		H		5d	10a	55	421	2.8	8.9	8.6 ± 0.2	62 ± 8	97 ± 6	24	22
18		H		5c	10a	52	407	2.2	8.8	8.9 ± 0.1	87 ± 8	79 ± 4	47	38
19		H		5b	10a	80	393	1.7	8.8	8.0 ± 0.3	33 ± 9	86 ± 8	120	57
20		H		5a	10a	52	378	1.3	8.7	7.4 ± 0.2	22 ± 5	64 ± 8	110	90
21		H		5h	10a	70	407	2.2	ND	7.9 ± 0.1	27 ± 4	87 ± 14	81	45
22		H		5i	10a	3	405	1.6	9	7.5 ± 0.1	16 ± 7	67 ± 11	120	85
23		H		5j	10a	64	419	2.7	ND	8.4 ± 0.1	66 ± 10	93 ± 13	29	26
24		H		5f	10a	3	419	2.5	8.9	8.5 ± 0.2	42 ± 19	96 ± 2	38	21
25		H		5e	10a	22	428	1.6	8.9	7.6 ± 0.1	18 ± 22	83 ± 6	95	35
26		H		5k	10a	29	446	1.9	ND	8.5 ± 0.1	54 ± 7	92 ± 7	99	35
27		F		5b	10b	51	410	2.1	8.5	8.8 ± 0.2	29 ± 12	74 ± 6	87	31
28		F		5c	10b	60	425	2.5	8.5	9.1 ± 0.1	39 ± 16	84 ± 6	28	16
29		F		5d	10b	62	439	3.4	8.6	8.6 ± 0.2	14 ± 12	88 ± 11	13	9
30		F		5i	10b	57	423	2.0	8.6	8.0 ± 0.1	25 ± 5	48 ± 8	87	35
31		F		5f	10b	3	437	2.8	8.5	8.7 ± 0.2	41 ± 16	94 ± 1	20	12
32		F		5g	10b	16	451	3.5	8.6	8.6 ± 0.2	24 ± 17	ND	5	5

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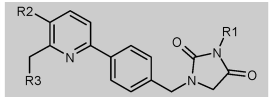
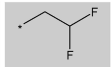
Table 2 Continued

No		Synthesis			Phys Chem			CB2	CB1	hERG	HLM	RLM
33	 F	5e	10b	67	446	1.9	9	8.6 ± 0.1	21 ± 19	83 ± 8	66	24
34	 H	5a	10c	43	435	3.4	8.7	7.9 ± 0.2	14 ± 8	91 ± 4	15	13
35	 F	5a	10d	21	453	3.9	8.4	8.6 ± 0.3	11 ± 5	ND	6	6
36	 H	5a	10e	80	439	3.7	7.4	9.4 ± 0.2	81 ± 10	84 ± 9	14	8
37	 F	5a	10f	39	457	4.1	7.2	9.4 ± 0.1	34 ± 15	91 ± 9	6	5
38	 F	5a	10g	68	441	3.4	6	8.1 ± 0.1	13 ± 9	57 ± 11	11	7
39	 H	5a	10h	55	457	4.5	5.3	8.7 ± 0.2	41 ± 10	29 ± 40	7	3
40	 F	5a	10i	36	475	4.7	5	9.3 ± 0.3	18 ± 5	42 ± 6	4	3
41	H	5a	10j	29	471	3	3.7	7.6 ± 0.2	16 ± 10	30 ± 8	27	29
42	 F	5a	10k	30	489	3	3	8.6 ± 0.2	16 ± 3	27 ± 8	16	13
43	 H	5b	15	29	392	5.6	ND	7.7 ± 0.1	61 ± 13	39 ± 6	40	7
44	 F	5i	10k	75	472	1.0	2.8	8.0 ± 0.1	30 ± 18	- 3 ± 4	> 70	> 70

potency, metabolic stability, and hERG affinity to predict the biological activity for these novel combinations. The generation of these COMFA models, validation, and prediction of the biological activity of ~3000 in silico compounds will be described by Klomp et al (in preparation). We have synthesized only the compounds for which the biological activity was predicted to be within the predefined criteria (CB2 pEC_{50} > 7.5; RLM $t_{1/2}$ > 20 min; hERG < 50% @ 10 μM). This resulted in the identification of **44**.

44 had good physicochemical properties, such as a MW = 473, log D = 1.0, pK_a = 2.8, and good solubility (89 mg/L). **44** was a potent cannabinoid CB2 receptor agonist (CB2 pEC_{50} = 8.0) with more than 1000-fold selectivity over the cannabinoid CB1 receptor. It lacked any affinity for the hERG ion channel (−3% @ 0.1 mM). The compound was metabolically stable (HLM/RLM $t_{1/2}$> 70 min, CL_{int} < 20 μL/min/mg) and was highly permeable in the Caco-2 model (P_{app} 173 nm/s). These favorable in vitro ADME parameters resulted in excellent in vivo pharmacokinetic properties as summarized in Table 1. **44** had a low clearance and 100% oral bioavailability. This compound was tested in the rat spinal nerve ligation model of neuropathic pain.[24] In this model, mechanical allodynia is induced by tight ligation of the left L5

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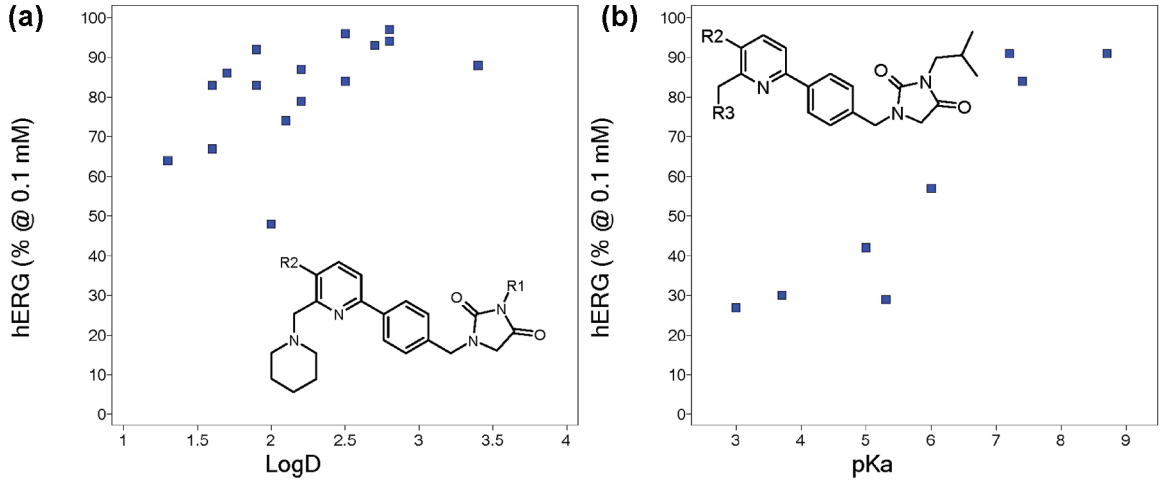

Figure 2. Correlation of hERG channel affinity (as measured by % displacement of ^{3}H-dofetilide with 10^{-4} M of test compound) with lipophilicity (a) and basicity (b) of 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione derivatives.

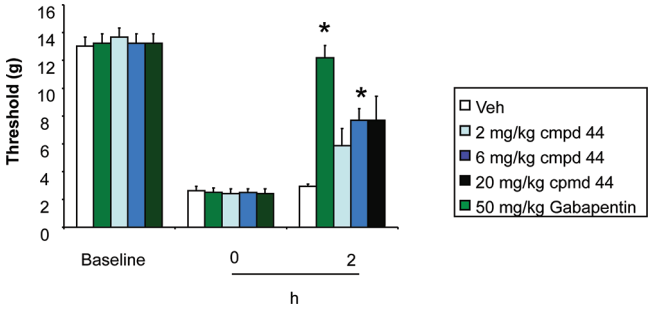

Figure 3. The effect of acute oral administration of compound **44** on neuropathy-induced mechanical allodynia in male wistar rats after dosing of 2, 6, and 20 mg/kg after 0 and 2 h. The reading at 0 min denotes the postsurgery withdrawal threshold (the difference between the presurgery and 0 min reading denotes the development of mechanical allodynia), this reading was followed by administration of **44**. Gabapentin (50 mg/kg, po) was included as a positive control. Data are expressed as mean ± SEM.

spinal nerve. This assay has been employed successfully to demonstrate antiallodynic effects of anticonvulsants (e.g., gabapentin), antidepressants (e.g., duloxetine), and opioid analgesics (e.g., tramadol), which are used clinically in the treatment of neuropathic pain.[1,2] **44** induced a dose-dependent antinociceptive effect after 2 h of dosing (Figure 3). Significant relieve of mechanical allodynia was observed at 6 mg/kg (po). High free plasma levels (>1900 nM) were required to observe significant antinociception. These levels were higher than expected based on the potency of the compound at the cannabinoid CB2 receptor, therefore we determined the brain penetration and its CSF levels. The total brain to total plasma ratio was 0.05 and the CSF/free plasma concentration ratio was 0.14, indicating active efflux out of the CNS. In the Caco-2 model, an A to B/B to A efflux ratio of 3.0 was observed, further suggesting that **44** may be subject to active transport. If the mechanism is centrally mediated, this may explain the high dose needed for efficacy in the spinal nerve ligation model, but other explanations cannot be ruled out at the moment. It is noteworthy that it was recently reported that the selective CB2 agonist GW842166 was not efficacious in patients with acute pain following third molar tooth extraction.[25] It is questioned whether the efficacy of selective cannabinoid CB2 receptor agonists is not due to residual cannabinoid CB1 receptor activity.[26,27] New series of imidazopyridine and decahydroquinoline amides CB2 agonists, which are highly selective, did not show any efficacy in a rat inflammatory pain model, whereas closely related, albeit less selective, analogues were active.[26,27] **44** is more than 1000-fold selective over the cannabinoid CB1 receptor, but it does have some residual cannabinoid CB1 receptor activity (30% @ 10 micro-Molar). Additional pharmacological profiling of **44** and other compounds from this novel chemical series will be described in future papers.

CONCLUSION

We have disclosed the 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione series as a novel chemotype possessing cannabinoid CB2 receptor activity. **44** was identified after a multiparameter optimization process in which lipophilicity and basicity were systematically varied to study their effect on metabolic stability, potency at the human cannabinoid CB2 receptor, and selectivity over the human cannabinoid CB1 receptor and the hERG channel. **44** is a potent and selective cannabinoid CB2 receptor agonist, which is orally bioavailable and has shown in vivo activity in a spinal nerve ligation model of neuropathic pain. The compound has low brain penetration, therefore it can be considered as a peripherally restricted agonist. This compound may be useful to study cannabinoid CB2 receptor biology in various preclinical models of human diseases such as pain, osteoporosis, and cancer.[8,9,21,28]

EXPERIMENTAL SECTION

All commercial chemicals and solvents are reagent grade and were used without further purification. The residues were purified by silica gel column (63−200 μm) chromatography or recrystallized from an appropriate solvent. The progress of reactions was monitored by thin layer chromatography (TLC) using commercially prepared silica gel 60 F_{254} glass-backed plates. Compounds were visualized under ultraviolet (UV) light. Purification performed on Gilson SemiPrep HPLC. Column: Luna 5 μm C18 (20), 100A, Axiapack, 150 mm × 21.20 mm, 5 μm. Detection: UV at 210 nm. Eluent A: Milli-Q water 900 mL + CH_3CN 100 mL + 0.05% HCOOH. Eluent B: CH_3CN 900 mL + MQ-water 100 mL + 0.05% HCOOH (Table 3). Flow: 20 mL/min .

Table 3

T (min)	A (%)	B (%)
0.0	90	10
6.00	0	90
30.00	0	90
31.00	0	100
34.00	0	100
35.00	90	10

Table 4

T (min)	A (%)	B (%)
0.0	98	02
5.00	0	100
5.70	0	100
5.71	98	02
7.00	98	02

The purity of all final compounds was >95% as determined with UPLC-MS; Waters Aquity Ultra Performance. Column: Acquity UPLC BEH C18, 2.1 mm × 100 mm, 1.7 μm. Detection: UV at 210 nm. Eluent A: MQ-water 900 mL + CH_3CN 100 mL + 0.05% HCOOH. Eluent B: CH_3CN 900 mL + MQ-water 100 mL + 0.05% HCOOH (Table 4). Flow: 0.60 mL/min.

^{1}H NMR was recorded on a Bruker Spectrospin 400 Ultrashield

General Procedures for Synthesis of 1-(4-Bromobenzyl)-3-alkyl-imidazolidine-2,4-dione Building Blocks. *Method 1*

(i) To a solution of 4-bromobenzaldehyde **2** (0.54 mol) and glycinamide hydrochloride (0.48 mol) methanol/water (1500 mL, 5.5/1) was added sodium hydroxide (0.54 mol). After stirring for 17 h at room temperature, the reaction mixture was cooled to 0 °C. Sodium borohydride (1.0 mol) was added, and the mixture was stirred until a clear solution was obtained. The reaction was quenched by addition of concentrated hydrochloric acid until pH = 3. After stirring for 17 h, the mixture was neutralized with a saturated aqueous solution of sodium hydrogen carbonate and the product was extracted into dichloromethane. The combined organic phases were washed with water, brine, dried over sodium sulfate, and concentrated under reduced pressure to afford 2-(4-bromo-benzylamino)-acetamide **3** (92 g).

(ii) To a solution of the product **3** obtained in the previous step (0.25 mol) in acetonitrile (1500 mL) were added CDI (0.5 mol) DMAP (0.5 mol). After stirring for 16 h at 60 °C, the solution was cooled to room temperature and poured into an aqueous solution of 2 M hydrochloric acid. The product was extracted into ethyl acetate and the combined organic phases were washed with water, brine, dried over sodium sulfate, and concentrated under reduced pressure. The remaining solid was stirred with acetone. Filtration afforded 1-(4-bromo-benzyl)-imidazolidine-2,4-dione **4** (45 g) as a white solid. The product was used in the following step without further purification.

(iii) To a solution of the product **4** obtained in the previous step (37.2 mmol) in DMF (90 mL) were added at room temperature, potassium carbonate (111 mmol) and the alkylbromide (74.3 mmol). After 17 h stirring at 50 °C under a nitrogen atmosphere, the reaction mixture was cooled to room temperature and filtered. The clear solution was concentrated under reduced pressure. Column chromatography afforded the corresponding 1-(4-Bromo-benzyl)-3-alkyl-imidazolidine-2,4-dione building block **5a**−**g** as a white solid.

*1-(4-Bromo-benzyl)-3-methyl-imidazolidine-2,4-dione (**5a**).* Overall yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, J = 8.61 Hz, 2H), 7.14 (d, J = 8.61 Hz, 2H), 4.52 (s, 2H), 3.73 (s, 2H), 3.06 (s, 3H).

*1-(4-Bromo-benzyl)-3-ethyl-imidazolidine-2,4-dione (**5b**).* Overall yield: 60%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, J = 8.22 Hz, 2H), 7.13 (d, J = 8.22 Hz, 2H), 4.52 (s, 2H), 3.71 (s, 2H), 3.59 (q, J = 7.43 Hz, 2H), 1.24 (t, J = 7.43 Hz, 3H).

*1-(4-Bromo-benzyl)-3-propyl-imidazolidine-2,4-dione (**5c**).* Overall yield: 63%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, J = 8.22 Hz, 2H), 7.13 (d, J = 8.22 Hz, 2H), 4.51 (s, 2H), 3.72 (s, 2H), 3.50 (dd J = 7.43 and 6.26 Hz, 2H), 1.72−1.60 (m, 2H), 0.93 (t, J = 7.43 Hz, 3H).

*1-(4-Bromo-benzyl)-3-isobutyl-imidazolidine-2,4-dione (**5d**).* Overall yield: 67%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, J = 8.22 Hz, 2H), 7.14 (d, J = 8.22 Hz, 2H), 4.50 (s, 2H), 3.72 (s, 2H), 2.15−2.05 (m, 1H), 1.50−1.42 (m, 2H), 0.93 (d, J = 6.65 Hz, 6H).

*1-(4-Bromo-benzyl)-3-(2,2-difluoro-ethyl)-imidazolidine-2,4-dione (**5e**).* Overall yield: 67%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51 (d, J = 8.22 Hz, 2H), 7.14 (d, J = 8.22 Hz, 2H), 6.21−5.87 (tt, J = 55.95, 4.70, and 4.30 Hz, 1H), 4.53 (s, 2H), 3.90 (td, J = 13.70 and 4.30 Hz, 2H), 3.80 (s, 2H).

*1-(4-Bromo-benzyl)-3-cyclopropylmethyl-imidazolidine-2,4-dione (**5f**).* Overall yield: 59%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, J = 8.22 Hz, 2H), 7.15 (d, J = 8.22 Hz, 2H), 4.53 (s, 2H), 3.74 (s, 2H), 3.56 (d, J = 7.44 Hz, 2H), 1.23−1.12 (m, 1H), 0.54−0.45 (m, 2H), 0.38−0.32 (m, 2H).

*1-(4-Bromo-benzyl)-3-cyclobutylmethyl-imidazolidine-2,4-dione (**5g**).* Overall yield: 56%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, J = 8.22 Hz, 2H), 7.13 (d, J = 8.22 Hz, 2H), 4.50 (s, 2H), 3.71 (s, 2H), 3.57 (d, J = 7.43 Hz, 2H), 2.75−2.62 (m, 1H), 2.08−1.97 (m, 2H), 1.92−1.70 (m, 4H).

Method 2

(i) To a solution of glycine hydrochloride (108 mmol) in water (40 mL) were added an aqueous solution of sodium hydroxide (108 mmol) and a solution of 4-bromobenzaldehyde **2** (108 mmol) in methanol (240 mL). After 30 min of stirring at room temperature, sodium borohydride (108 mmol) was added portionwise to this suspension. After 18 h of stirring at room temperature, the reaction mixture was concentrated under reduced pressure and the resulting aqueous phase was washed with diethyl ether. The aqueous phase was neutralized by the addition of an aqueous solution of 2 M hydrochloric acid. The resulting precipitate was collected by filtration and washed with water and diethyl ether. Drying the white solid afforded (4-bromo-benzylamino)-acetic acid **6** (y = 71%). The product was used in the following step without further purification.

(ii) To a suspension of the product **6** obtained in the previous step (6.96 mmol) in dichloromethane (20 mL) were added triethylamine (13.9 mmol), an alkylamine (7.66 mmol), and *o*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (PyBOP) (8.36 mmol). After 17 h of stirring at room temperature, the reaction mixture was concentrated under reduced pressure. Column chromatography afforded 2-(4-bromo-benzylamino)-*N*-alkyl-acetamide **7h**−**k**.

(iii) To a solution of the product **7h**−**k** obtained in the previous step (6.96 mmol) in acetonitrile were added (diimidazol-1-yl)ketone (13.9 mmol) and 4-dimethylaminopyridine (13.9 mmol). After 17 h of stirring at 60 °C, the reaction mixture was cooled to room temperature and quenched by addition of a saturated aqueous solution of sodium hydrogen carbonate. The product was extracted into ethylacetate and the combined organic phases were washed with water and brine and dried over sodium sulfate. Column chromatography afforded the title compound 1-(4-bromo-benzyl)-3-alkyl-imidazolidine-2,4-dione **5h**−**k** as a light-yellow oil.

*1-(4-Bromo-benzyl)-3-isopropyl-imidazolidine-2,4-dione (**5h**).* Overall yield: 36%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, J = 8.61 Hz, 2H), 7.13 (d, J = 8.61 Hz, 2H), 4.49 (s, 2H), 4.38−4.30 (m, 1H), 1.43 (d, J = 6.65 Hz, 6H).

*1-(4-Bromo-benzyl)-3-cyclopropyl-imidazolidine-2,4-dione (**5i**).* Overall yield: 46%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (d, J = 8.61 Hz, 2H), 7.14 (d, J = 8.61 Hz, 2H), 4.48 (s, 2H), 3.66 (s, 2H), 2.65−2.56 (m, 1H), 0.97 (d, J = 5.87 Hz, 4H).

*1-(4-Bromo-benzyl)-3-cyclobutyl-imidazolidine-2,4-dione (**5j**).* Overall yield: 57%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.49 (d, J = 8.22 Hz, 2H), 7.14 (d, J = 8.22 Hz, 2H), 4.60−4.51 (m, 1H), 4.49 (s, 2H), 3.65 (s, 2H), 2.95−2.82 (m, 2H), 2.23−2.13 (m, 2H), 1.91−1.81 (m, 1H), 1.79−1.65 (m, 1H).

*1-(4-Bromo-benzyl)-3-(2,2,2-trifluoro-ethyl)-imidazolidine-2,4-dione (**5k**).* Overall yield: 25%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52(d, J = 8.61 Hz, 2H), 7.15 (d, J = 8.61 Hz, 2H), 4.55 (s, 2H), 4.16 (q, J = 8.61 Hz, 2H), 3.83 (s, 2H).

General Procedure for Synthesis of 2-Bromo-pyridine Building Blocks. To a solution of 6-bromo-pyridine-2-carbaldehyde **8a** or **8b** (135 mmol) in dichloromethane (500 mL) was slowly added an appropriate cyclic amine (149 mmol) at 10 °C. After stirring for 15 min at 10 °C, acetic acid (149 mmol) was added, followed by the portionwise addition of sodium triacetoxyborohydride, while the temperature was kept at 5−10 °C. After stirring for 2 h at room temperature, the reaction mixture was poured into a saturated aqueous solution of sodium hydrogen carbonate. The product was extracted into dichloromethane, and the combined organic phases were washed with brine, dried over sodium sulfate, and concentrated under reduced pressure. Column chromatography (heptane/ethylacetate 8/2) afforded the corresponding 2-bromo-pyridine **10a**−**k** as a colorless oil.

*2-Bromo-6-piperidin-1-ylmethyl-pyridine (**10a**).* Yield: 93%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54−7.43 (m, 2H), 7.33 (d, J = 7.43 Hz, 1H), 3.60 (s, 2H), 2.48−2.38 (m, 4H), 1.63−1.54 (m, 4H), 1.48−1.39 (m, 2H).

*6-Bromo-3-fluoro-2-piperidin-1-ylmethyl-pyridine (**10b**).* Yield: 80%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (dd, J = 8.22 and 3.52 Hz, 1H), 7.26 (d, J = 8.61 and 8.22 Hz, 1H), 3.69 (d, J = 2.74 Hz, 2H), 2.54−2.46 (m, 4H), 1.61−1.53 (m, 4H), 1.45−1.36 (m, 2H).

*2-Bromo-6-(3-methyl-piperidin-1-ylmethyl)-pyridine (**10c**).* Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47−7.37 (m, 2H), 7.26 (d, J = 7.43 Hz, 1H), 3.54 (s, 2H), 2.76−2.64 (m, 2H), 1.92 (td, J = 10.96 and 3.52 Hz, 1H), 1.68−1.44 (m, 5H), 0.87−0.72 (m, 4H).

*6-Bromo-3-fluoro-2-(3-methyl-piperidin-1-ylmethyl)-pyridine (**10d**).* Yield: 38%. (m/z) = 288 $(M + H)^+$.

*2-Bromo-6-(3-fluoro-piperidin-1-ylmethyl)-pyridine (**10e**).* Yield: 24%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 (dd, J = 7.83 and 7.43 Hz, 1H), 7.47 (d, J = 7.43 Hz, 1H), 7.36 (d, J = 7.83 Hz, 1H), 4.76−4.56 (m, 1H), 3.68 (s, 2H), 2.82−2.71 (m, 1H), 2.60−2.47 (m, 2H), 2.43−2.36 (m, 1H), 1.93−1.78 (m, 2H), 1.73−1.50 (m, 2H).

*6-Bromo-3-fluoro-2-(3-fluoro-piperidin-1-ylmethyl)-pyridine (**10f**).* Yield: 55%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (dd, J = 8.61 and 3.52 Hz, 1H), 7.28 (d, J = 8.61 and 8.22 Hz, 1H), 4.72−4.53 (m, 1H), 3.78 (bs, 2H), 2.97−2.86 (m, 1H), 2.60−2.57 (m, 1H), 2.55−2.47 (m, 1H), 2.42−2.35 (m, 1H), 1.92−1.75 (m, 2H), 1.62−1.47 (m, 2H).

*4-(6-Bromo-3-fluoro-pyridin-2-ylmethyl)-morpholine (**10g**).* Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (dd, J = 8.22 and 3.52 Hz, 1H), 7.28 (d, J = 8.61 and 8.22 Hz, 1H), 3.73−3.69 (m, 6H), 2.61−2.54 (m, 4H).

*2-Bromo-6-(3,3-difluoro-piperidin-1-ylmethyl)-pyridine (**10h**).* Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58−7.49 (m, 2H), 7.47 (d, J = 7.43 Hz, 1H), 3.74 (s, 2H), 2.71 (dd, J = 11.35 and 10.96 Hz, 2H), 2.54 (dd, J = 5.48 and 5.09 Hz, 2H), 1.97−1.84 (m, 2H), 1.83−1.76 (m, 2H).

*6-Bromo-2-(3,3-difluoro-piperidin-1-ylmethyl)-3-fluoro-pyridine (**10i**).* Yield: 65%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41 (dd, J = 8.22 and 3.52 Hz, 1H), 7.29 (d, J = 8.61 and 8.22 Hz, 1H), 3.86 (d, J = 2.35 Hz, 2H), 2.79 (dd, J = 11.35 and 10.95 Hz, 2H), 2.59 (dd, J = 5.48 and 5.09 Hz, 2H), 1.91−1.72 (m, 4H).

*4-(6-Bromo-pyridin-2-ylmethyl)-thiomorpholine 1,1-dioxide (**10j**).* Yield: 90%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61−7.54 (dd, J = 7.83 and 7.43 Hz, 1H), 7.42 (d, J = 7.83 Hz, 1H), 7.39 (d, J = 7.43 Hz, 1H), 3.81 (s, 2H), 3.14−3.04 (m, 8H).

*4-(6-Bromo-3-fluoro-pyridin-2-ylmethyl)-thiomorpholine 1,1-dioxide (**10k**).* Yield: 77%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (dd, J = 8.22 and 3.52 Hz, 1H), 7.32 (d, J = 8.61 and 8.22 Hz, 1H), 3.89 (d, J = 2.74 Hz, 2H), 3.17 (m, 8H).

*2-Bromo-6-cyclohexylmethyl-pyridine (**15**)*

(i) A solution of 2,6-dibromopyridine **11** (5.8 mmol) in THF/hexane/diethyl ether (1/1/3, 15 mL) was added dropwise, under a nitrogen atmosphere, to a solution of a 2.5 M n-butyllithium in hexane (2.43 mL, 6.08 mmol) at −78 °C . After 10 min of stirring, a solution of cyclohexanecarbonitrile **12** (5.8 mmol) in THF/hexane/diethyl ether (1/1/3, 4 mL) was added and the reaction mixture was stirred for 2.5 h at −78 °C. The reaction mixture was warmed to room temperature and stirred for another 1.5 h. The reaction mixture was quenched by the addition of an aqueous solution of 2 M sulfuric acid (7 mL). After vigorous stirring for 2 h, water was added and the product was extracted into diethyl ether. The combined organic phases were washed with a saturated aqueous solution of sodium hydrogen carbonate, brine, dried over sodium sulfate, and concentrated under reduced pressure. Column chromatography afforded (6-bromo-pyridin-2-yl)-cyclohexyl-methanone **13** (800 mg) as a clear oil.

(ii) A suspension of the product **13** obtained in the previous step (2.24 mmol) and 4-methylbenzenesulfonyl hydrazide (2.46 mmol) in ethanol (2 mL) was heated to 100 °C for 15 min in a microwave. After cooling to room temperature, the reaction mixture concentrated under reduced pressure. Column chromatography afforded N'-((6-bromopyridin-2-yl)(cyclohexyl)methylene)-4-methylbenzenesulfonohydrazide **14** (608 mg) as a white solid.

(iii) To a solution of the product **14** obtained in the previous step (1.38 mmol) in 4 mL dichloromethane was added slowly to a solution of 20% diisobutylaluminiumhydride in toluene (13.8 mmol). After 17 h of stirring at room temperature, the reaction mixture was quenched by adding slowly an aqueous solution of 2 M sodium hydroxide until pH = 10. The product was extracted into ethylacetate, and the combined organic phases were washed with water, brine, dried over sodium sulfate, and concentrated under reduced pressure. Column chromatography afforded the title compound 2-bromo-6-cyclohexylmethyl-pyridine **15** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42−7.21 (m, 3H), 3.62 (d, J = 7.04 Hz, 2H), 1.81−0.85 (m, 11H).

General Method for Synthesis of 1-(4-(Pyridin-2-yl)benzyl)imidazolidine-2,4-dion Derivatives

(i) To a solution of the 1-(4-bromobenzyl)-imidazolidine-2,4-dione derivative **5a**−**k** (6.2 mmol), bis(pinacolato)diboron (6.2 mmol) and potassium acetate (18.5 mmol) in DMF (50 mL) under a nitrogen atmosphere was added 1,1′-bis(diphenylphosphino)ferrocenedichloro palladium(II) (0.18 mmol). After 17 h of stirring at 75 °C, the reaction mixture was cooled to room temperature. Water was added, and the product was extracted into ethyl acetate. The combined organic phases were washed with a saturated aqueous solution of sodium hydrogen carbonate, water, and brine, dried over sodium sulfate, and concentrated under reduced pressure to afford the corresponding boronic ester **16a**−**k** as a black oil. The product was used in the following step without further purification.

(ii) To a solution of the product **16a**−**k** obtained in the previous step (3.52 mmol) and an appropriate 2-bromopyridine derivative **10a**−**k** or **15** (2.93 mmol) in toluene/ethanol (4/1, 25 mL) was added an aqueous solution of 2 M potassium carbonate. After 15 min of stirring under a nitrogen atmosphere,

tetrakis(triphenylphosphine)palladium(0) (0.073 mmol) was added and this mixture was stirred for 17 h at 75 °C under a nitrogen atmosphere. After completion, the mixture was cooled to room temperature and filtered through decalite. Water was added to the filtrate, and the product was extracted into ethylacetate. The combined organic phases were washed with water and brine, dried over sodium sulfate, and concentrated under reduced pressure. Column chromatography on silicagel (*n*-heptane/ethylacetate) afforded the corresponding 1-(4-(pyridin-2-yl)benzyl)-imidazolidine-2,4-dion final products **1**, **18**−**44**.

*3-Isobutyl-1-(4-(6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**1**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, J = 8.22 Hz, 2H), 7.72 (dd, J = 8.22 and 7.83 Hz, 1H), 7.56 (d, J = 8.22 Hz, 1H), 7.43 (d, J = 7.83 Hz, 1H), 7.34 (d, J = 8.22 Hz, 2H), 4.62 (s, 2H), 3.74 (s, 2H), 3.72 (s, 2H), 3.36 (d, J = 7.43 Hz, 2H), 2.53−2.47 (m, 4H), 2.15−2.05 (m, 1H), 1.65−1.58 (m, 4H), 1.50−1.42 (m, 2H), 0.93 (d, J = 6.65 Hz, 6H).

*1-(4-(6-(Piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-3-propylimidazolidine-2,4-dione (**18**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.22 Hz, 2H), 7.22 (dd, J = 7.83 and 7.04 Hz, 1H), 7.56 (d, J = 7.83 Hz, 1H) 7.43 (d, J = 7.04 Hz, 1H), 7.34 (d, J = 8.22 Hz, 2H), 4.61 (s, 2H), 3.73 (s, 2H), 3.71 (s, 2H), 3.51 (t, J = 7.43 Hz, 2H), 2.53−2.47 (m, 4H), 1.73−1.56 (m, 6H), 1.50−1.42 (m, 2H), 0.94 (t, J = 7.43, 3H).

*3-Ethyl-1-(4-(6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**19**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.22 Hz, 2H), 7.74 (dd, J = 7.83 and 7.43 Hz, 1H), 7.61 (d, J = 7.83 Hz, 1H), 7.45 (d, J = 7.43 Hz, 1H), 7.35 (d, J = 8.22 Hz, 2H), 4.61 (s, 2H), 3.72 (bs, 4H), 3.61 (q, J = 7.04 Hz, 2H), 2.53−2.47 (m, 4H), 1.65−1.57 (m, 4H), 1.50−1.43 (m, 2H), 1.25 (t, 3H).

*3-Methyl-1-(4-(6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**20**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.22 Hz, 2H), 7.72 (dd, J = 7.83 and 7.04 Hz, 1H), 7.56 (d, J = 7.83 Hz, 1H), 7.43 (d, J = 7.04 Hz, 1H), 7.35 (d, J = 8.22 Hz, 2H), 4.62 (s, 2H), 3.75 (s, 2H), 3.72 (s, 2H), 3.06 (s, 3H), 2.50 (bs, 4H), 1.65−1.57 (m, 4H), 1.50−1.43 (m, 2H).

*3-Isopropyl-1-(4-(6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**21**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.61 Hz, 2H), 7.72 (dd, J = 7.83 and 7.43 Hz, 1H), 7.56 (d, J = 7.83 Hz, 1H), 7.43 (d, J = 7.43 Hz, 1H), 7.34 (d, J = 8.61 Hz, 2H), 4.58 (s, 2H), 4.40−4.32 (m, 1H), 3.72 (s, 2H), 3.67 (s, 2H), 2.50 (bs, 4H), 1.66−1.57 (m, 6H), 1.44 (d, J = 7.04 Hz, 6H).

*3-Cyclopropyl-1-(4-(6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**22**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, J = 8.22 Hz, 2H), 7.72 (dd, J = 7.83 and 7.43 Hz, 1H), 7.56 (d, J = 7.83 Hz, 1H), 7.43 (d, J = 7.43 Hz, 1H), 7.34 (d, J = 8.22 Hz, 2H), 4.59 (s, 2H), 3.72 (s, 2H), 3.67 (s, 2H), 2.66−2.60 (m, 1H), 2.54−2.46 (m, 4H), 1.65−1.57 (m, 2H), 1.50−1.42 (m, 2H), 1.00−0.96 (m, 4H).

*3-Cyclobutyl-1-(4-(6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**23**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, J = 8.22 Hz, 2H), 7.72 (dd, J = 7.83 and 7.43 Hz, 1H), 7.56 (d, J = 7.83 Hz, 1H), 7.43 (d, J = 7.43 Hz, 1H), 7.34 (d, J = 8.22 Hz, 2H), 4.63−4.53 (m, 3H), 3.72 (s, 2H), 3.67 (s, 2H), 2.97 −2.84 (m, 2H), 2.54−2.46 (m, 4H), 2.24−2.14 (m, 2H), 1.92−1.82 (m, 1H), 1.79−1.67 (m, 1H), 1.65−1.57 (m, 4H), 1.52−1.40 (m, 2H).

*3-(Cyclopropylmethyl)-1-(4-(6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**24**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.22 Hz, 2H), 7.72 (dd, J = 8.22 and 7.83 Hz, 1H), 7.56 (d, J = 8.22 Hz, 1H), 7.43 (d, J = 7.83 Hz, 1H), 7.35 (d, J = 8.22 Hz, 2H), 4.62 (s, 2H), 3.75 (s, 2H), 3.72 (s, 2H), 3.41 (d, J = 7.04 Hz, 2H), 2.53−2.47 (m, 4H), 1.654−1.58 (m, 4H), 1.52−1.42 (m, 2H), 1.24−1.14 (m, 1H), 0.56−0.47 (m, 2H), 0.40−0.33 (m, 2H).

*3-(2,2-Difluoroethyl)-1-(4-(6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**25**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, J = 8.61 Hz, 2H), 7.73 (dd, J = 7.83 and 7.42 Hz, 1H), 7.56 (d, J = 7.83 Hz, 1H), 7.44 (d, J = 7.43 Hz, 1H), 7.35 (d, J = 8.61 Hz, 2H), 6.05 (Tt, J = 55.6 and 4.30 Hz, 1H), 4.63 (s, 2H), 3.92 (Td, J = 13.7 and 4.30 Hz, 2H), 3.81 (s, 2H), 3.72 (s, 2H), 2.54−2.40 (m, 4H), 1.67−1.56 (m, 4H), 1.50−1.41 (m, 2H).

*1-(4-(6-(Piperidin-1-ylmethyl)pyridin-2-yl)-benzyl)-3-(2,2,2-trifluoroethyl)-imidazolidine-2,4-dione (**26**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (d, J = 8.22 Hz, 2H), 7.73 (dd, J = 7.83 and 7.43 Hz, 1H), 7.56 (d, J = 7.83 Hz, 1H), 7.44 (d, J = 7.43 Hz, 1H), 7.35 (d, J = 8.22 Hz, 2H), 4.64 (s, 2H), 4.17 (q, J = 8.61 Hz, 2H), 3.84 (s, 2H), 3.72 (s, 2H), 2.54−2.46 (m, 4H), 1.67−1.56 (m, 4H), 1.51−1.42 (m, 2H).

*3-Ethyl-1-(4-(5-fluoro-6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**27**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, J = 8.22 Hz, 2H), 7.63 (dd, J = 8.61 and 3.52 Hz, 1H), 7.43 (dd, J = 9.00 and 8.61 Hz, 1H), 7.35 (d, J = 8.61 Hz, 2H), 4.61 (s, 2H), 3.85 (d, J = 2.35 Hz, 2H), 3.74 (s, 2H), 3.61 (q, J = 7.43 Hz, 2H), 2.62 (bs, 4H), 1.66−1.57 (m, 4H), 1.47−1.38 (m, 2H), 1.25 (t, J = 7.43, 3H).

*1-(4-(5-Fluoro-6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-3-propylimidazolidine-2,4-dione (**28**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.61 Hz, 2H), 7.63 (dd, J = 8.61 and 3.52 Hz, 1H), 7.43 (dd, J = 9.00 and 8.61 Hz, 1H), 7.34 (d, J = 8.61 Hz, 2H), 4.61 (s, 2H), 3.74 (s, 2H), 3.51 (t, J = 7.04 Hz, 2H), 3.49 (s, 2H), 2.70−2.51 (m, 4H), 1.76−1.55 (m, 6H), 1.49−1.35 (m, 2H), 0.94 (t, J = 7.04, 3H).

*1-(4-(5-Fluoro-6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-3-isobutylimidazolidine-2,4-dione (**29**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.61 Hz, 2H), 7.62 (dd, J = 8.21 and 3.13 Hz, 1H), 7.43 (t, J = 8.61 Hz, 1H), 7.34 (d, J = 8.21 Hz, 2H), 4.62 (s, 2H), 3.83 (d, J = 1.96 Hz, 2H), 3.75 (s, 2H), 3.37 (d, J = 7.43 Hz, 2H), 2.62−2.54 (m, 4H), 2.15−2.04 (m, 1H), 1.65−1.57 (m, 4H), 1.46−1.37 (m, 2H), 0.93 (d, J = 6.65 Hz, 6H).

*3-Cyclopropyl-1-(4-(5-fluoro-6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**30**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.22 Hz, 2H), 7.62 (dd, J = 8.61 and 3.52 Hz, 1H), 7.42 (dd, J = 8.61 and 8.22 Hz, 1H), 7.34 (d, J = 8.22 Hz, 2H), 4.58 (s, 2H), 3.82 (d, J = 2.74 Hz, 2H), 3.68 (s, 2H), 2.67−2.55 (m, 5H), 1.64−1.54 (m, 4H), 1.44−1.84 (m, 2H), 1.83−1.75 (m, 2H), 1.64−1.57 (m, 4H), 1.47−1.37 (m, 2H), 1.00−0.95 (m, 4H).

*3-(Cyclopropylmethyl)-1-(4-(5-fluoro-6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**31**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97 (d, J = 8.22 Hz, 2H), 7.62 (dd, J = 8.61 and 3.52 Hz, 1H), 7.42 (t, J = 8.61 Hz, 1H), 7.35 (d, J = 8.22 Hz, 2H), 4.62 (s, 2H), 3.82 (d, J = 2.74 Hz, 2H), 3.76 (s, 2H), 3.41 (d, J = 7.01 Hz, 2H), 2.61−2.55 (m, 4H), 1.64−1.56 (m, 4H), 1.45−1.38 (m, 2H), 1.23−1.16 (m, 1H), 0.55−0.48 (m, 2H), 0.39−0.34 (m, 2H).

*3-(Cyclobutylmethyl)-1-(4-(5-fluoro-6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**32**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.22 Hz, 2H), 7.62 (dd, J = 8.61 and 3.52 Hz, 1H), 7.42 (t, J = 8.61 Hz, 1H), 7.33 (d, J = 8.22 Hz, 2H), 4.60 (s, 2H), 3.82 (d, J = 2.74 Hz, 2H), 3.73 (s, 2H), 3.58 (d, J = 7.43 Hz, 2H), 2.76−2.66 (m, 1H), 2.63−2.53 (m, 4H), 2.09−1.99 (m, 2H), 1.93−1.84 (m, 2H), 1.83−1.75 (m, 2H), 1.64−1.57 (m, 4H), 1.47−1.37 (m, 2H).

*3-(2,2-Difluoroethyl)-1-(4-(5-fluoro-6-(piperidin-1-ylmethyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**33**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, J = 8.61 Hz, 2H), 7.62 (dd, J = 8.61 and 3.52 Hz, 1H), 7.43 (dd, J = 9.00 and 8.61 Hz, 1H), 7.37 (d, J = 8.61 Hz, 2H), 4.05 (Tt, J = 55.95 and 4.30 Hz, 1H), 4.63 (s, 2H), 3.92 (Td, J = 13.69 and 4.30 Hz, 2H), 3.83 (s, 2H), 3.82 (s, 2H), 2.63−2.55 (m, 4H), 1.65−1.55 (m, 4H), 1.45−1.37 (m, 2H).

*3-Isobutyl-1-(4-(6-((3-methylpiperidin-1-yl)-methyl)-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**34**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.22 Hz, 2H), 7.73 (dd, J = 7.83 and 7.43 Hz, 1H), 7.57 (d, J = 7.83 Hz, 1H), 7.44 (d, J = 7.43 Hz, 1H), 7.34 (d, J = 8.22 Hz, 2 H), 4.63 (s, 2H), 3.74 (s, 2H), 3.73 (s, 2H), 3.37 (d, J = 7.43 Hz, 2H), 2.93−2.82 (m, 2H), 2.16−1.99 (m, 2H), 1.79−1.52 (m, 6H), 0.93 (d, J = 7.04 Hz, 6H), 0.87 (d, J = 5.87 Hz, 3H).

*1-(4-(5-Fluoro-6-((3-methylpiperidin-1-yl)-methyl)-pyridin-2-yl)-benzyl)-3-isobutylimidazolidine-2,4-dione (**35**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.22 Hz, 2H), 7.62 (dd, J = 8.61 and 3.52 Hz, 1H), 7.43 (dd, J = 9.00 and 8.61 Hz, 1H), 7.34 (d, J = 8.22 Hz, 2H), 4.62 (s, 2H), 3.83 (bs, 2H), 3.76 (s, 2H), 3.37 (d, J = 7.43 Hz, 2H), 3.06−2.94 (m, 2H), 2.15−2.02 (m, 2H), 1.81−1.55 (m, 6H), 0.93 (d, J = 7.04 Hz, 6H), 0.85 (d, J = 5.87 Hz, 3H).

*1-(4-(6-((3-Fluoropiperidin-1-yl)-methyl)-pyridin-2-yl)-benzyl)-3-isobutylimidazolidine-2,4-dione (**36**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.22 Hz, 2H), 7.74 (dd, J = 7.83 and 7.43 Hz, 1H), 7.58 (d, J = 7.83 Hz, 1H), 7.42 (d, J = 7.43 Hz, 1H), 7.35 (d, J = 8.22 Hz, 2 H), 4.62 (s, 2H), 3.76 (s, 2H), 3.75 (s, 2H), 3.37 (d, J = 7.43 Hz, 2H), 2.79−2.68 (m, 2H), 2.54−2.43 (m, 2H), 2.15−1.84 (m, 6H), 0.93 (d, J = 6.65 Hz, 6H).

*1-(4-(5-Fluoro-6-((3-fluoropiperidin-1-yl)-methyl)-pyridin-2-yl)-benzyl)-3-isobutylimidazolidine-2,4-dione (**37**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.22 Hz, 2H), 7.64 (dd, J = 8.61 and 3.52 Hz, 1H), 7.44 (d, J = 9.00 and 8.61 Hz, 1H), 7.35 (d, J = 8.22 Hz, 2H), 4.62 (s, 2H), 3.86 (d, J = 2.35 Hz, 2H), 3.76 (s, 2H), 3.37 (d, J = 7.43 Hz, 2H), 2.84−2.75 (m, 2H), 2.64−2.54 (m, 2H), 2.17−1.85 (m, 6H), 0.93 (d, J = 6.65 Hz, 6H).

*1-(4-(5-Fluoro-6-morpholin-4-ylmethyl-pyridin-2-yl)-benzyl)-3-isobutylimidazolidine-2,4-dione (**38**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.22 Hz, 2H), 7.62 (dd, J = 8.61 and 3.52 Hz, 1H), 7.42 (t, J = 8.62 Hz, 1H), 7.34 (d, J = 8.22 Hz, 2H), 4.62 (s, 2H), 3.82 (d, J = 2.74 Hz, 2H), 3.75 (s, 2H), 3.36 (d, J = 7.43 Hz, 2H), 2.62−2.55 (m, 4H), 2.14−2.05 (m, 1H), 1.64−1.57 (m, 4H), 1.46−1.37 (m, 2H), 0.93 (d, J = 6.65 Hz, 6H).

*1-(4-(6-((3,3-Difluoropiperidin-1-yl)-methyl)-pyridin-2-yl)-benzyl)-3-isobutylimidazolidine-2,4-dione (**39**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.61 Hz, 2H), 7.75 (dd, J = 7.83 and 7.43 Hz, 1H), 7.57 (d, J = 7.83 Hz, 1H), 7.44 (d, J = 7.43 Hz, 1H), 7.35 (d, J = 8.61 Hz, 2 H), 4.62 (s, 2H), 3.85 (s, 2H), 3.75 (s, 2H), 3.37 (d, J = 7.43 Hz, 2H), 2.78 (t, J = 11.35 Hz, 2H), 2.63−2.54 (m, 2H), 2.17−2.04 (m, 1H), 1.97−1.77)m, 4H), 0.93 (d, J = 6.65 Hz, 6H).

*1-(4-(6-((3,3-Difluoropiperidin-1-yl)-methyl)-5-fluoropyridin-2-yl)-benzyl)-3-isobutylimidazolidine-2,4-dione (**40**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (d, J = 8.22 Hz, 2H), 7.66 (dd, J = 8.61 and 3.52 Hz, 1H), 7.46 (dd, J = 9.00 and 8.61 Hz, 1H), 7.35 (d, J = 8.22 Hz, 2H), 4.62 (s, 2H), 3.99 (d, J = 2.35 Hz, 2H), 3.76 (s, 2H), 3.37 (d, J = 7.43 Hz, 2H), 2.88 (t, J = 11.35 Hz, 2H), 2.69−2.62 (m, 2H), 2.17−2.05 (m, 1H), 1.92−1.74)m, 4H), 0.93 (d, J = 6.65 Hz, 6H).

*1-(4-(6-((1,1-Dioxo-1λ6-thiomorpholin-4-yl)-methyl)-pyridin-2-yl)-benzyl)-3-isobutyl-imidazolidine-2,4-dione (**41**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, J = 8.61 Hz, 2H), 7.77 (dd, J = 7.83 and 7.43 Hz, 1H), 7.63 (d, J = 7.83 Hz, 1H), 7.36 (m, 3H), 4.63 (s, 2H), 3.92 (s, 2H), 3.76 (s, 2H), 3.37 (d, J = 7.43 Hz, 2H), 3.18−3.09 (m, 8H), 2.15−2.04 (m, 1H), 0.93 (d, J = 6.65 Hz, 6H).

*1-(4-(6-(1,1-Dioxo-1λ6-thiomorpholin-4-ylmethyl)-5-fluoro-pyridin-2-yl)-benzyl)-3-isobutyl-imidazolidine-2,4-dione (**42**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, J = 8.22 Hz, 2H), 7.68 (dd, J = 8.61 and 3.52 Hz, 1H), 7.48 (dd, J = 9.00 and 8.61 Hz, 1H), 7.36 (d, J = 8.22 Hz, 2H), 4.62 (s, 2H), 4.02 (d, J = 2.35 Hz, 2H), 3.76 (s, 2H), 3.37 (s, 2H), 3.24−3.18 (m, 4H), 3.13−3.06 (m, 4H), 2.14−2.04 (m, 1H), 0.93 (d, J = 7.04 Hz, 6H).

*1-(4-(6-(Cyclohexylmethyl)-pyridin-2-yl)-benzyl)-3-ethylimidazolidine-2,4-dione (**43**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.22 Hz, 2H), 7.64 (dd, J = 7.83 and 7.43 Hz, 1H), 7.50 (d, J = 7.83 Hz, 1H), 7.35 (d, J = 8.22 Hz, 2H), 7.05 (d, J = 7.43 Hz, 1H), 4.62 (s, 2H), 3.73 (s, 2H), 3.61 (q, J = 7.43 Hz, 2H), 2.72 (d, J = 7.04 Hz, 2H), 1.92−1.78 (m, 1H), 1.76−1.58 (m, 6H), 1.31−1.15 (m, 5H), 1.10−0.98 (m, 2H).

*3-Cyclopropyl-1-(4-(6-(1,1-dioxo-1λ6-thiomorpholin-4-ylmethyl)-5-fluoro-pyridin-2-yl)-benzyl)-imidazolidine-2,4-dione (**44**).* ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (d, J = 8.22 Hz, 2H), 7.68 (dd, J = 7.83 and 7.43 Hz, 1H), 7.63 (dd, J = 9.00 and 3.52 Hz, 1H), 7.36 (d, J = 8.22 Hz, 2H), 4.59 (s, 2H), 4.02 (s, 2H), 3.70 (s, 2H), 3.24−3.19 (m, 4H), 3.13−3.07 (m, 4H), 2.67−2.59 (m, 1H), 1.01−0.96 (m, 4H).

Determination of eLogD and pK_a. The physicochemical properties eLogD and pK_a were experimentally determined as described previously[29,30]

Agonist-Induced cAMP Change in Human CB2 Tranfected CHO Cells. Adenylate cyclase assays were carried out using CHO cells stably overexpressing the human recombinant cannabinoid CB2 receptor. Cells were cultured in DMEM/HAMF12 containing 1% (v/v) penicillin/streptomycin (Gibco 15140−122), 10% FBS Biowitthaker (DE-14-701-F), and 400 μg/mL Geneticin (Invitrogen 10131−027). Test compounds and reference cannabinoid CB receptor agonist CP55,940 were dissolved in DMSO, and dilutions were made in serum-free medium containing 10 μM Rolipram (Sigma R6520) and 2 μM Forskolin (Sigma, F3917). Then 10 μL of each dilution was transferred to an assay plate (384-well white culture plate, Perkin-Elmer). Cell suspensions containing 106 cells/mL in DMEM/HAMF12 containing 1% (v/v) penicillin/streptomycin were prepared from hCB2_C2-CHO cells and 10 μL (10000 cells/well) thereof was transferred to the assay plate and cells were incubated for 45 min at 37 °C. Homogeneous time-resolved fluorescence (HTRF; CisBio) was used as a read-out by sequentially adding 10 μL of cAMP-XL665 and 10 μL of anti-cAMP(Eu) cryptate; after 1 h incubation at room temperature, fluorescence at 615 nm and 665 nm was measured on Envision (Perkin-Elmer). Results were calculated from the 665 nm/615 nm ratios obtained for individual test compounds and were compared to values obtained for the reference compound.

Agonist-Induced cAMP Change in Human CB1 Tranfected CHO Cells. Adenylate cyclase assays were carried out using CHO cells stably overexpressing the human recombinant cannabinoid CB1 receptor. Cells were cultured in DMEM/HAMF12 containing 1%(v/v) penicillin/streptomycin (Gibco 15140-122), 10% FBS Biowitthaker (DE-14-701-F), 400 μg/mL Geneticin (Invitrogen 10131-027), and Zeocine 250 μg/mL (Invitrogen, 45-0430). Test compounds and reference cannabinoid CB agonist (CP55,940) were dissolved in DMSO, and dilutions were made in serum-free medium containing 10 μM Rolipram (Sigma R6520) and 2 μM Forskolin (Sigma, F3917). Then 10 μL of each dilution was transferred to an assay plate (384-well white culture plate, Perkin-Elmer). Cell suspensions containing 106 cells/mL in DMEM/HAMF12 containing 1% (v/v) penicillin/streptomycin were prepared from hCB1_A2-CHO cells and 10 μL (10000 cells/well) thereof was transferred to the assay plate and cells were incubated for 45 min at 37 °C. Homogeneous time-resolved fluorescence (HTRF; CisBio) was used as a read-out by sequentially adding 10 μL of cAMP-XL665 and 10 μL of anti-cAMP(Eu) cryptate; after 1 h incubation at room temperature, fluorescence at 615 nm and 665 nm was measured on Envision (Perkin-Elmer). Results were calculated from the 665 nm/615 nm ratios obtained for individual test compounds and were compared to values obtained for the reference compound.

In Vitro Determination of Affinity at the hERG Channel Expressed in HEK293 Cells. This assay is used to evaluate test compounds for their inhibition of [^{3}H]-dofetilide binding to hERG channels stably expressed in HEK293 cells (HEK.HERG). The class III antiarrhythmic dofetilide has a high affinity for the hERG channel. Compounds that display a high affinity for hERG and displace [^{3}H]-dofetilide are considered to have an increased probability of producing cardiotoxicity. For this study, compounds were prepared in glass vials and stored at −20 °C as 10^{-2} M stocks. The amount of radiolabel remaining on the filters was then determined by scintillation counting. The highest DMSO concentration in the assay was equal to 1%. Test compounds were assayed in a 6 pt log dose response concentration range from 10^{-4} M to 10^{-11} M.

Caco-2 Permeability. Test compound was added to either apical (A) or basolateral (B) chamber at a final concentration of 20 μM in PBS, pH7.4. After 1 h of incubation at 37 °C, an aliquot from the opposite side

of the monolayer was removed and terminated in acetonitrile. The compounds were quantified by LC-MS/MS. Monolayer integrity for each well was evaluated by lucifer yellow permeability (as determined by fluorescence). The efflux ratio was defined as the B to A/A to B apparent permeability (P_{app}) ratio.

Plasma Protein Binding. Plasma protein binding was assessed by equilibrium dialysis using Dianorm apparatus (Germany) with plasma and buffer (PBS, pH 7.4) separated by a 5000 Da cutoff membrane (Harvard Apparatus, Edenbridge, UK). The assay was performed for 6 h at 37 °C with compound initially at 10 μM in plasma. The concentrations in plasma and buffer at the end of the incubation were determined by LC-MS/MS following protein precipitation.

Microsomal Stability. The rate of metabolism was assessed by incubation at 37 °C at pH 7.4 with hepatic microsomes (0.5 mg protein/mL) supplemented with 1 mM NADPH and an initial substrate concentration of 1 μM. Substrate depletion over a time course was measured by LC-MS/MS following protein precipitation.

Pharmacokinetics in Rat. Test compounds were administered via jugular vein cannulae (iv) or by oral gavage (po) to groups of four nonfasted male Wistar BRL rats. Serial blood samples were taken into EDTA-containing tubes from each rat at various time-points to 24 h. Plasma was harvested by centrifugation (3000 rcf) with storage at −20 °C until bioanalysis. Samples were analyzed following protein precipitation by LC-MS/MS with quantitation against a standard curve prepared with authentic standards. Time−concentration profiles were analyzed using WinNonlin v 4.1 (Pharsight, USA) to derive PK parameters.

Serial CSF Sampling. Male Wistar BRL rats were surgically prepared with implantation of a cannula for CSF sampling from the cisterna magna. Three days after surgery, four rats were dosed with test compound at 20 mg/kg by oral gavage. Serial CSF samples (30 μL) and tail vein blood samples were taken at 0.5, 1, 2, and 4 h after dosing. Additionally, brains were harvested at 4 h and also at 2 h in a nonsurgically prepared satellite group. Plasma was separated from blood and analyzed as described for rat PK studies. Brain samples were homogenized with 3 volumes of ice-cold PBS, pH 7.4, and were protein precipitated prior to analysis. CSF samples were quantified against standards prepared in artificial CSF. Analysis was by LC-MS/MS for plasma, brain and CSF samples.

The Rat Spinal Nerve Ligation (Chung) Model of Neuropathic Pain. Male Wistar rats (228−301 g body weight at time of surgery) were employed in the study. Rats were placed on an elevated (∼40 cm) mesh floor in perspex boxes and the rats' withdrawal threshold to a mechanical stimulus (calibrated von Frey filaments) was measured using filaments of increasing force (2.6−167 mN) as described below. The von Frey filaments were applied to the plantar surface of the paw and threshold response determined using the up and down method. A positive response was noted if the paw was sharply withdrawn. A cutoff of 15 g was selected as the upper limit for testing. Following baseline measurements, each animal was anaesthetised and the L5 spinal nerve tightly ligated. The animals were allowed to recover from the surgery for a period of at least three days. On the day of drug administration, the paw withdrawal thresholds were remeasured (0 min). Immediately after this reading, the rats were dosed orally with vehicle or test compound and paw withdrawal measurements were taken again at various time points after compound administration. Data are expressed as values ± SEM. Statistical analysis was performed using the Kruskal−Wallis one-way analysis of variance, a nonparametric statistical test. Each of the treatment groups was then compared against the vehicle group using the nonparametric Dunn's test.

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ABBREVIATIONS USED

CB1, Cannabinoid 1 receptor; CB2, Cannabinoid 2 receptor; hERG, Human Ether-à-go-go Related Gene; TRPV1, Transient receptor potential vanilloid 1; IFN γ, Interferon gamma; ALS, Amyotrophic lateral sclerosis; CHO, Chinese hamster ovary; CL_{int}, Intrinsic clearance; CL, Clearance; PyBO, Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; COMFA, Comparative Molecular Field Analysis; MW, Molecular weight; HLM, Human liver microsomes; RLM, Rat liver microsomes; ADME, Absorption, distribution, metabolism, excretion; CI, Confidence interval; CSF, Cerebrospinal fluid; CDI, 1,1′-Carbonyldiimidazole; DMAP, 4-Dimethylaminopyridine; DMF, Dimethylformamide; M, Molar; THF, Tetrahydrofuran; DMEM, Dulbecco's modified Eagle's medium; FBS, Fetal bovine serum; DMSO, Dimethyl sulfoxide; PBS, Phosphate buffered saline; NADPH, Nicotinamide adenine dinucleotide phosphate; EDTA, Ethylenediaminetetraacetic acid; SEM, Standard error of the mean; V_{ss}, Volume of distribution (steady state); F, Bioavailability; f_u, fraction unbound

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