Bioorganic & Medicinal Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

The discovery and optimization of benzimidazoles as selective $Na_V1.8$ blockers for the treatment of pain

Alan D. Brown[a,*], Sharan K. Bagal[a], Paul Blackwell[b], David C. Blakemore[d], Bruce Brown[b], Peter J. Bungay[c], Martin Corless[b], James Crawforth[b], David Fengas[e], David R. Fenwick[b], Victoria Gray[b], Mark Kemp[b], Wolfgang Klute[b], Laia Malet Sanz[b], Duncan Miller[b], Yoshihisa Murata[b], C. Elizabeth Payne[c], Sarah Skerratt[a], Edward B. Stevens[c], Joseph S. Warmus[d,*]

[a] Pfizer Medicine Design, Pfizer Ltd., The Portway Building, Granta Park, Cambridge CB21 6GS, UK
[b] Pfizer Medicinal Sciences, Pfizer Global R&D, Sandwich CT13 9FF, UK
[c] Medicinal Sciences, Pfizer Ltd., The Portway Building, Granta Park, Cambridge CB21 6GS, UK
[d] Pfizer Medicine Design, Groton Laboratories, Eastern Point Road, Groton, CT 06340, USA
[e] Concept Life Sciences, Discovery Park, Ramsgate Road, Sandwich, Kent CT13 9FF, UK

ARTICLE INFO

Keywords:
Benzimidazole
PF-06305591
Neuropathic pain
Inflammatory pain
Voltage gated sodium channel
$Na_V1.8$
SCN10A

ABSTRACT

The voltage gated sodium channel $Na_V1.8$ has been postulated to play a key role in the transmission of pain signals. Core hopping from our previously reported phenylimidazole leads has allowed the identification of a novel series of benzimidazole $Na_V1.8$ blockers. Subsequent optimization allowed the identification of compound **9**, PF-06305591, as a potent, highly selective blocker with an excellent preclinical *in vitro* ADME and safety profile.

1. Introduction

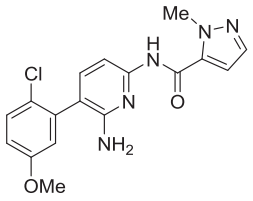
Voltage-gated sodium channels (Na_V) are a family of transmembrane (TM) ion channel proteins. Structurally, they are members of the 6-TM ion channel family and are composed of a transmembrane α-subunit of approximately 260 kDa with associated transmembrane β-subunits of lower molecular weight. The family is comprised of nine members, $Na_V1.1$ – $Na_V1.9$ which can be subdivided into tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) subtypes. Na_V's play a key role in controlling excitability of neurons by regulating the threshold of firing, underlying the upstroke of the action potential and controlling the duration of interspike interval.[1] Selective block of Na_V channels as pain targets has gained traction following the recognition that some Na_V subtypes show preferential or exclusive expression in peripheral sensory neurons.[2] A number of pre-clinical studies have implicated $Na_V1.3$, 1.7, 1.8 and 1.9, which are expressed in dorsal root ganglion neurons (DRGs) and trigeminal neurones, in nociceptive processing.[2] $Na_V1.8$ is highly (but not exclusively) expressed in nociceptors and its expression and function is modulated by agents that cause pain. Genetic ablation of $Na_V1.8$ in rodents results in deficits in nociception following inflammation, but not neuropathic pain while recent human genetic evidence suggests that gain of function mutations in $Na_V1.8$ contribute to painful peripheral neuropathy.[3] A-803467 was one of the first compounds in the public domain that demonstrated selectivity across human Na_V subtypes and attenuated pain sensitivity in models of both nerve injury and inflammation induced pain, providing the first pharmacological evidence supporting a role for $Na_V1.8$ in both inflammatory and neuropathic pain.[4]

We have previously reported PF-04531083 as a selective $Na_V1.8$ blocker (Figure 1).[5] As part of our follow on program in this area we were keen to identify a clinical candidate which would allow us to investigate significantly higher IC_{50} multiples of $Na_V1.8$ blockade. To this

Abbreviations: ADMET, absorption, distribution, metabolism, excretion and toxicity; AUC, area under the concentration versus time curve; Cl_p, plasma clearance; Cl_u, unbound clearance; F, bioavailability; HLM, human liver microsomes; hERG, human ether–a–go–go; hHeps, human hepatocytes; IC_{50}, half-maximum inhibitory concentration; PK, pharmacokinetics; PMC, parallel medicinal chemistry; RRCK, Ralph Russ canine kidney cell line; $T_{1/2}$, half-life; TPSA, topological surface area; V_d, volume of distribution

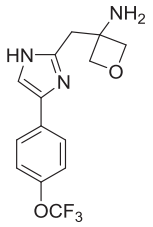
* Corresponding authors at: Pfizer Global Research and Development, 445 Eastern Point Road, 220/235A, Groton, CT 06340, USA.
E-mail address: Joseph.Warmus@pfizer.com (J.S. Warmus).

https://doi.org/10.1016/j.bmc.2018.12.002
Received 22 August 2018; Received in revised form 20 November 2018; Accepted 1 December 2018



PF-04531083

$hNa_V1.8$ IC_{50} 190 nM
$hNa_V1.1$, 1.5 & 1.7 IC_{50} > 30 μM
hERG IC_{50} > 30 μM
clogP 3.1, logD > 3.6, LiPE < 3.1
pKa 3.4
Solubility 0.7 μg/ml (pH 7.2)



1

$hNa_V1.8$ IC_{50} 57 ± 15 nM
$hNa_V1.1$, 1.5 & 1.7 IC_{50} > 17 μM
hERG IC_{50} 10 μM
clogP 3.1, LiPE 4.1
pKa 6.4
Solubility > 300 μg/ml (pH 7.2)

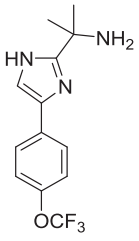
Figure 1. Previously reported Pfizer NaV1.8 blockers.[5,6]

end our aim was to identify compounds which were not only more potent $Na_V1.8$ blockers but also had improved solubility over PF-04531083, whilst retaining its low *in vivo* clearance and excellent ion channel selectivity profile. Our initial efforts in this area led to the identification of phenylimidazole **1**[6] (Figure 1) which demonstrated excellent $Na_V1.8$ potency and selectivity, as well as improved solubility and lipophilic efficiency (LipE = -Log(h $Na_V1.8$ IC_{50})-clogP) over PF-04531083. However, achieving acceptable selectivity over the IKr (hERG) channel proved to be particularly problematic in this series.

2. Design and synthesis of Nav 1.8 inhibitors

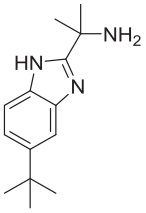
Screening of (available) compounds, based on the pharmacophoric understanding developed in our work around compound **1**, identified benzimidazole **3** as a potential new lead (Figure 2). Direct comparison with compound **2** from our phenylimidazole series[6] showed that, whilst **3** had retained good $Na_V1.5$ selectivity, this new lead had reduced potency/LipE and only moderate hERG selectivity. In spite of this profile, we were encouraged by the fact that analogues of **3** could be readily prepared using existing in-house library protocols which would allow a rapid assessment of the suitability of this new series for further optimization.

A library of 5-*t*-butyl-2-aminomethylbenzimidazoles was therefore prepared to establish the potential of this new lead series. A typical library protocol suitable to prepare these compounds is illustrated in Scheme 1. The general route to the substituted imidazoles began with 4-(*tert*-butyl)benzene-1,2-diamine. Condensation with a Boc protected amino acid using HATU and an amine base in DMF led to a mixture of regioisomeric amides. This was concentrated and condensed using acetic acid to provide the desired benzimidazole. Deprotection using either 4 M HCl in dioxane or a TFA methylene chloride mixture gave the



2

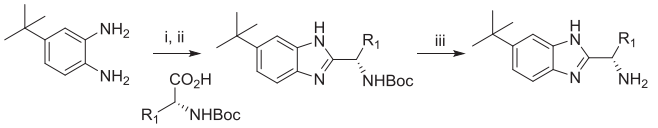
$hNa_V1.8$ IC_{50} 53 ± 23 nM
$hNa_V1.5$ IC_{50} 9.7 μM
hERG IC_{50} 2.6 μM
clogP 2.9, LiPE 4.4



3

$Na_V1.8$ IC_{50} 607 ± 105 nM
$Na_V1.5$ 12% @ 10 μM
hERG IC_{50} 41 μM
clogP 3.0, LipE 3.2

Figure 2. Comparison of benzimidazole lead **3** with representative phenyl imidazole **2**.



Scheme 1. General PMC route to benzimidazoles. Reagents and Conditions: i) HATU, DIPEA or Et_3N, DMF, 25 °C. ii) HOAc, 25 °C. iii) 4 M HCl, dioxane, CH_2Cl_2, or TFA, CH_2Cl_2, 25 °C.

desired products.

Structure Activity Relationship (SAR) data from compounds prepared using this synthetic approach established rapidly that this was an attractive new lead series (Table 1). Specifically, $hNa_V1.8$ IC_{50} < 50 nM potency could be attained in compounds with relatively low clogP, giving LipE values approaching 6 (see for example compounds **7**, **9** and **15**).[7] Other points worthy of note are the need for a basic centre in the R substituent (note the inactivity of compound **5**) and the fact that there was significant scope for varying this substituent whilst retaining good levels of $Na_V1.8$ blockade.

Initial profiling of compound **9**, one of the leads from this initial synthetic effort, showed it to have a highly attractive profile with respect to Na_V selectivity, hERG activity, passive permeability (Papp AB) and *in vitro* metabolic stability (Figure 3).

Having established that these benzimidazoles represented an attractive new series we then investigated the potential for further optimization. A range of compounds containing potential *t*-butyl replacements were prepared using synthetic approaches similar to that outlined in Scheme 1. Representative examples are described in Table 2.

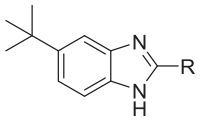
Intriguingly, CF_3O-, which represented our optimal substituent in this region in the previously reported phenylimidazole series gave a significantly weaker $Na_V1.8$ blocker in this new series (compound **17** versus compound **14**).[6] Other replacements were inferior to *t*-butyl with respect to $Na_V1.8$ blockade, although several (e.g. **18**, **19**, **21** and **24**) had similarly high LipE values to our best leads, suggesting that it may be possible to prepare attractive compounds with alternative substituents to *t*-butyl at this position.

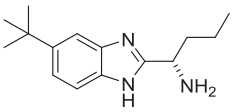
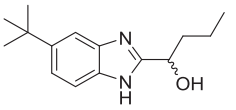
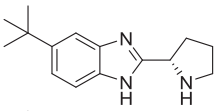
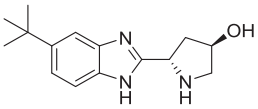
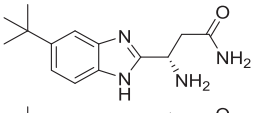
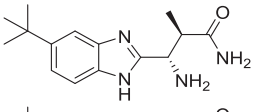
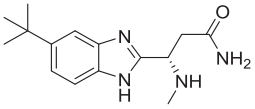
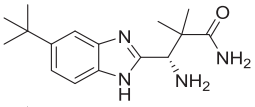
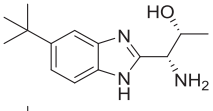
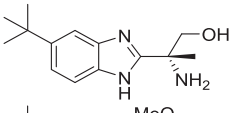
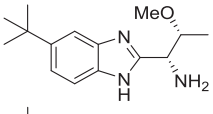
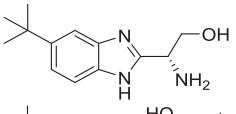
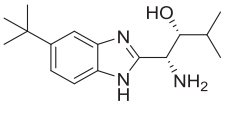
At this point we switched our attention to investigating alternative heterocyclic cores/benzimidazole replacements. Quinoxalines **27** and **29** were prepared as direct analogues of compound **15**, as illustrated in Scheme 2. Neither **27**, **29** nor their respective enantiomers (**28**, **30**) showed significant $Na_V1.8$ blockade (IC_{50}'s > 1 μM), suggesting that a [6,5] fused heterocycle and/or a group containing an heterocyclic NH (mimicking the benzimidazole NH) was required for high levels of $Na_V1.8$ inhibitory activity.

Following on from this observation we prepared imidazopyridine **46** (Scheme 3) as an analogue containing a [6,5] fused heterocycle which has good pharmacophoric overlap with our existing benzimidazole core (as in compound **9**). The synthesis of compound **46** started by condensation of *tert*-butylaminopyridine **40** with ethyl bromopyruvate to prepare the imidazopyridine core. Conversion to aldehyde **42** went smoothly, followed by preparation of the non-racemic *tert*-butylsufinamide **43** by condensation of the aldehyde with (*S*)-(-)-2-methyl-2-propanesulfinamide using Cs_2CO_3 as a dehydrating agent.[8] The addition of the enolate of *p*-tolyl propionate to sulfinamide **43** provided a 28% yield of the undesired syn product and a 9% yield of a 1:2 syn:anti mixture after chromotography. This 2:1 mixture was carried on through an amidation using ammonium hydroxide, followed by removal of the sulfinamide to obtain the desired amine. The diastereomers were separated using supercritical fluid chromatography using a chiral column to provide the desired imidazopyridine **46**.

As shown in Figure 4, compound **46** is a potent $Na_V1.8$ blocker with similar activity to the corresponding benzimidazole, **9**. Examination of ACD labs calculated pKas for compound **46** suggested that the first protonated form of this compound would involve protonation of the (non-bridgehead) ring N, resulting in a system which would mimic the

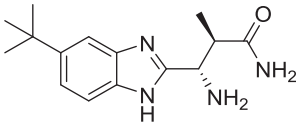
Table 1
2-Position SAR of 5-*t*-Butylbenzimidazoles.



No.	Structure	$Na_V1.8$ IC_{50} (nM), ± SEM	LipE	clogP
4		2, ± 4	3.9	3.7
5		> 1000	< 2.3	3.7
6		43, ± 0.7	4.2	3.2
7		31, ± 4	5.6	1.9
8		130, ± 28	5.0	1.9
9		15, ± 2	6.3	1.5
10		742, ± 61	4.6	1.7
11		797, ± 111	4.2	1.9
12		59, ± 7	5.4	1.8
13		65, ± 9	5.4	1.8
14		24, ± 3	5.1	2.5
15		106, ± 37	5.6	1.4
16		227, ± 27	4.0	2.7

imidazole NH of compound **9** (Figure 5). Taken together with the lack of activity of compounds **27** and **29**, these data supported the argument that the presence of a suitably positioned NH in the heterocyclic core is crucial for potent $Na_V1.8$ blockade in systems of this type.

Following on from this work and more detailed profiling of a range

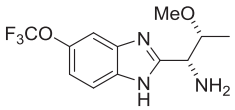
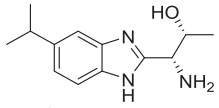
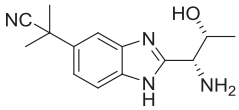
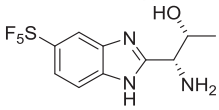
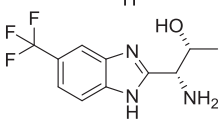
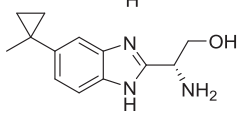
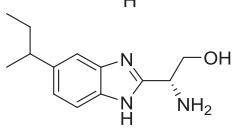
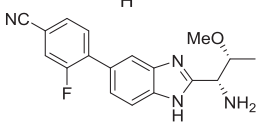
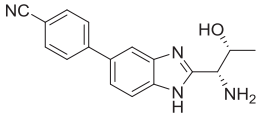
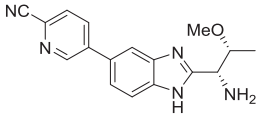


9

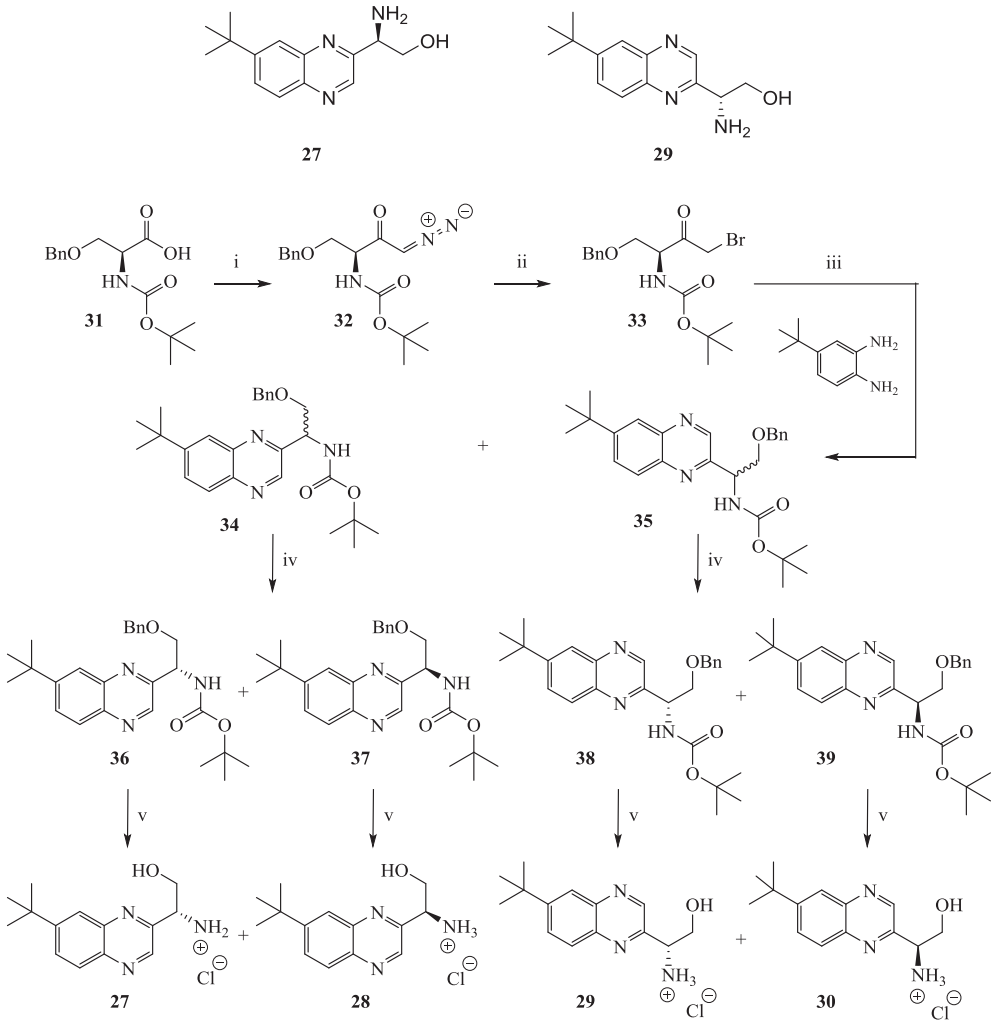
$Na_V1.8$ IC_{50} 15 ± 2 nM
$Na_V1.1$, 1.2 and 1.5 IC_{50} >30 μM
hERG IC_{50} >30 μM
clogP 1.5, LipE 6.3
P_{app} AB[a] 3.5
HLM[b] <11, hHeps[c] <2

Figure 3. Structure and initial data on key benzimidazole compound 9. [a]Papp AB, 10^{-6}cm/*sec*, RRCK cell line; [b]Human Liver Microsomal Stability, μL/min/mg protein; [b]Human Hepatocyte Stability, μL/min/million cells.

Table 2
Profiles of Potential 5-*t*-Butyl Replacements.

No.	Structure	$Na_V1.8$ IC_{50} (nM), ± SEM	LipE	clogP
17		2567, ± 199	3.5	2.1
18		43, ± 10	6.0	1.4
19		728, ± 63	5.5	0.6
20		95, ± 25	4.7	2.3
21		534, ± 103	5.4	0.9
22		593, ± 154	5.1	1.1
23		708, ± 149	4.6	1.6
24		18, ± 2	5.5	2.2
25		579, ± 0.4	5.0	1.2
26		998, ± 126	5.1	0.9

of compounds, we concluded that compound **9** (PF-06305591) had the most attractive *in vitro* profile of the compounds prepared. Not only was **9** significantly more soluble than our earlier Na_V blocker PF-04531083



Scheme 2. Preparation of Quinoxalines 27–30. Reagents and Conditions: i) 1) isopropyl chloroformate, Et_3N, THF, 0 °C. 2) CH_2N_2, 0 °C (87%). ii) HBr, Et_2O, 0 °C (64%). iii) $HClO_4$•SiO_2, CH_3CN, 25 °C (37%). iv) separation of isomers. v) $HCl_{(aq.)}$, 0–25 °C (73% – 78%).

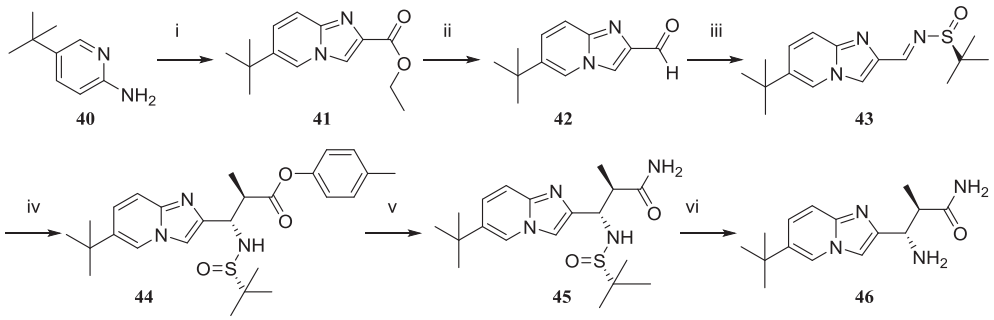
(Figure 1) but it was also exquisitely selective across the other human Na_V isoforms and across a representative set of additional ion channels (Figure 6). In addition, wide ligand (CEREP™) profiling and follow-up screening showed no significant off-target activity (< 20% activity at 10 μM). We therefore set about preparing multi-gram quantities of this compound for further *in vivo* profiling.

3. Multi-gram synthesis of PF-06305591

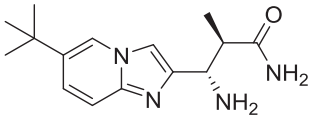
Given the lack of a convenient route to homochiral α-methylasparagine (or a synthetic equivalent), an alternative synthetic approach to that outlined in Scheme 1 was developed. Key to this alternative synthesis (as outlined in Scheme 4) was the introduction of the desired *anti*-stereochemistry by utilizing the stereocontrol inherent in the alkylation of **51** to provide the known *trans* methyl azetidinone **52**.[9] This was coupled to 4-*tert*-butyldiaminobenzene to give a mixture of regioisomeric amides, which upon condensation provided desired benzimidiazole **54**. The imide of the lactam was prepared by reacting **54** with Boc anhydride, thus allowing the lactam to be opened by ammonia, followed by removal of the Boc to provide desired compound **9**.

4. In vivo PK studies for PF-06305591

With sufficient material in hand, rat pharmacokinetic studies were carried out on compound **9** (Table 3). Intravenous (i.v.) and oral (p.o.) dosing in rats was conducted at 1 mg/kg and 3 mg/kg respectively.



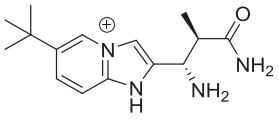
Scheme 3. Preparation of imidazopyridine 31. Reagents and Conditions: i) Ethyl bromopyruvate, EtOH, 90 °C (58%). ii) DIBAL, CH_2Cl_2, -78 °C (98%). iii) (*S*)-(-)-2-Methyl-2-propanesulfinamide, Cs_2CO_3, CH_2Cl_2, 25 °C (72%). iv) *p*-Tolyl propionate, NaHMDS, -78 °C, THF (9%). v) NH_4OH, DMF, 25 °C (100%). vi) HCl, *i*PrOH, MeOH, 25 °C.



46

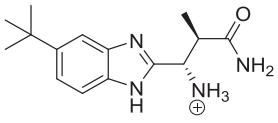
$Na_V1.8$ IC_{50} 45 ± 4 nM
$Na_V1.5$ IC_{50} >10 μM
hERG IC_{50} >72 μM
clogP 1.3, LipE 6.1
P_{app} AB[a] 2.2
HLM[a] <11, hHeps[b] 2.5

Figure 4. Structure and *in vitro* profile of compound **46**. [a]Papp AB, 10^{-6}cm/*sec*, RRCK cell line; [b]Human Liver Microsomal Stability, μL/min/mg protein; [b]Human Hepatocyte Stability, μL/min/million cells.



46

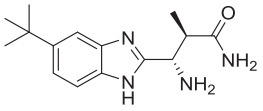
Calculated pKa (heterocycle) 7.4
Calculated pKa (NH_2) 3.9
Measured pKa 7.0, 3.0



9

Calculated pKa (heterocycle) 4.5
Calculated pKa (NH_2) 7.3
Measured pKa 5.9, 1.9

Figure 5. Comparison of calculated and measured first protonated form of **46** with compound **9**.



(9), PF-06305591
MWt 274; clogP 1.5; LogD 2.1
TPSA 98; pKa 5.9
Solubility 2 mg/ml (pH 7.4)
$hNa_V1.8$ IC_{50} 15 nM; LipE 6.3
$hNa_V1.1$, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 IC_{50} >30 μM

Target	IC_{50} (μM)
KVLQT1 (KCNQ1, KV7.1)	>30
KV1.5 (Cardiac)	>30
KV1.1/1.2 (CNS)	>30
SK2 (KCNN2) (CNS)	>30
T-type Ca (Cardiac/CNS)	>30
L-Type (Cardiac)	>30
hERG (Cardiac)	>30
GABA-A (α1 g2)	>30

Figure 6. Structure and full *in vitro* data package for compound **9**. [a]Human Liver Microsomal Stability, μL/min/mg protein; [b]Human Hepatocyte Stability, μL/min/million cells.

Compound **9** was shown to have good rat bioavailability, which, coupled with its excellent *in vitro* human metabolic profile and excellent solubility, suggested significant potential as a back-up to our previous clinical candidate, PF-04531083.

5. Conclusions

In summary, core hopping from our previously reported phenyl imidazole NaV1.8 blockers has allowed the identification of a novel series of benzimidazole $Na_V1.8$ blockers with high LipE. Within this series exquisite pan-Na_V and general off-target selectivity was successfully achieved, along with excellent solubility and *in vitro* (human) metabolic stability. Lead compound **9** (PF-06305591) has the potential to be a back-up candidate to our previously reported clinical candidate, PF-04531083. With an enhanced solubility profile relative to PF-04531083, **9** (PF-06305591) offers the possibility of investigating higher IC_{50} multiples of Nav1.8 blockade in the clinic, and therefore a more thorough evaluation of the role of $Na_V1.8$ in the treatment of pain.

6. Experimental section

6.1. General experimental

The following section contains the experimental of the final compounds in this paper. Full experimental for all intermediates including spectra, as well as details of general methods, HPLC conditions and abbreviations, can be found in the Supporting Information.

Compound **9** (PF-06305591) and PF-04531083 are commercially available via MilliporeSigma (catalog numbers PZ0297 and PZ0273 respectively).

All procedures performed on animals were in accordance with regulations and established guidelines and were reviewed and approved by an Institutional Animal Care and Use Committee or thorough and ethical review process.

6.2. 2-(5-(tert-Butyl)-1H-benzo[d]imidazol-2-yl)propan-2-amine (3).

2-(5-(*tert*-Butyl)-1H-benzo[*d*]imidazol-2-yl)propan-2-amine was prepared in parallel format using the following protocol: A solution of 2-((*tert*-butoxycarbonyl)amino)-2-methylpropanoic acid in DMA (100 umol, 1.0 eq) was dispensed into 8 vials, followed by a solution of PyBOP (55 mg, 110 umol, 1.1 eq). The vials were diluted with DMA (800 μL), capped and shaken at 30 °C for 30 min. A solution of the intermediates, including 4-(*tert*-butyl)benzene-1,2-diamine (100 umol, 1.0 eq), in DMA (400 ul) was dispensed to the vials. DIEA (75 μL, 300 umol, 1.0 eq) was added to the vials, which were capped and shaken at 30 °C for 16 hrs. The solvent was removed by a Speedvac to give the crude intermediate *tert*-butyl (1-((2-amino-4-(*tert*-butyl)phenyl)amino)-2-methyl-1-oxopropan-2-yl) carbamate which was used for next step without further purification. The intermediates, including *tert*-butyl (1-((2-amino-4-(*tert*-butyl)phenyl)amino)-2-methyl-1-oxopropan-2-yl)carbamate (75 μmol, 1.0 eq) in acetic acid (400 μL) were dispensed into in 8 mL vials followed by addition of 6 N HCl (150 μL) to each vial. The vials were capped and shaken at 100 °C for 2 hrs. The solvent was removed by a Speedvac and the residue was purified by preparative HPLC to give pure 2-(5-(*tert*-butyl)-1H-benzo[*d*]imidazol-2-yl)propan-2-amine. HRMS for $C_{14}H_{22}N_3$ MS *m/z* $[M + H]^+$: Calc'd: 232.1808, Found: 232.1808.

6.3. (1S)-1-(5-(tert-butyl)-2,3-dihydro-1H-benzo[d]imidazol-2-yl)butan-1-amine (4)

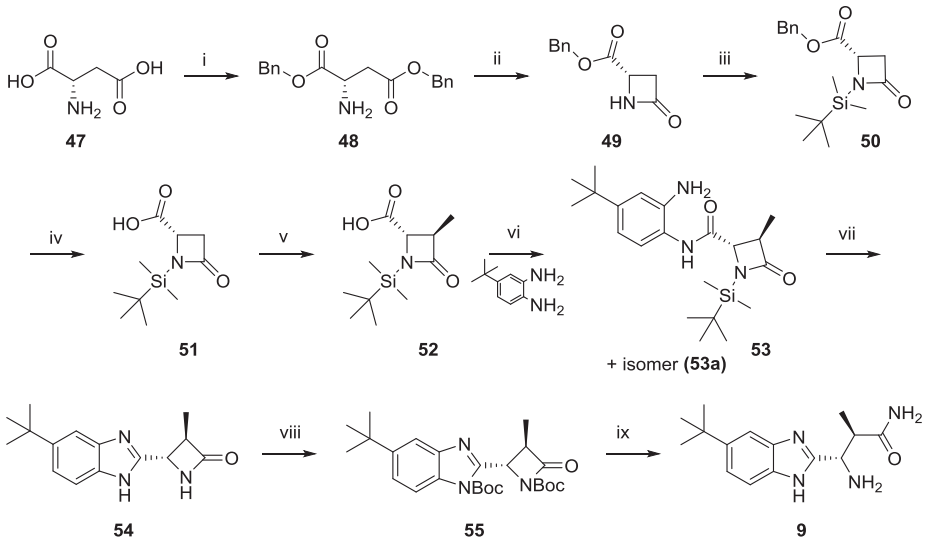
(R)-5-(*tert*-butyl)-2-(pyrrolidin-2-yl)-1*H*-benzo[*d*]imidazole (6)

(3R,5R)-5-(5-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)pyrrolidin-3-ol (7)

(R)-3-amino-3-(5-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propanamide (8)

(1R,2R)-1-(5-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)-2-methoxypropan-1-amine (14)

The title compounds were prepared in a parallel fashion by the following protocol: Step 1: To a 0.325 M solution of amino acid in DMF



Scheme 4. Preparation of **9** (PF-06305591). Reagents and Conditions: i) BnOH, TsOH, toluene, 110Â°C (79%). ii) 1) TMSCl, Et_2O, -78 °C-25 °C. 2) *t*BuMgBr, Et_2O, -78 °C-25 °C (40%). iii) TBDMSCl, Et_3N, DMF (93%). iv) 10% Pd/C, H_2, EtOH, 25 °C (72%). v) LDA, THF, -78 °C-20 °C, MeI, -20 °C-25 °C (64%). vi) HATU, DMF, 0–25 °C (97%). vii) AcOH, 35 °C (95%). viii) Boc_2O, CH_3CN, DMAP, 0–25 °C (90%). ix) 1) 0.88 M NH_3, DMF, 25 °C (73%). 2) HCl, 25 °C (76%).

Table 3
Rat pharmacokinetic data for compound 9, PF-06305591.

Property	PF-06305591 (9)
Rat PK	
Cl_p (mL/min/kg)	40.0
Cl_u (mL/min/kg)	214
$T_{1/2}$ (h)	1.5
AUC0-∞ (ng.h/mL)	962
V_d (L/kg)	2.3
F (%)	61%

(400 µL, 125 µmol, 1 eq) was added a 0.325 M solution of 4-*tert*-butyl-1,2-diaminobenzene in DMF (400 µL, 125 µmol, 1 eq), a 0.325 M solution of HATU in DMF (400 µL, 125 µmol, 1 eq) and triethylamine (35 µL, 250 5 µmol, 2 eq). The reaction was shaken at 60 °C for 16 h before concentrating *in vacuo*. To the residue was added HOAc (1.25 mL) and the reaction shaken at 80 °C for 3 h. The reaction was cooled, concentrated *in vacuo* and purified using preparative HPLC to afford the benzimidazole intermediate. Step 2: To the benzimidazole was added a solution of TFA/DCM (1:5, 2 mL) and the reaction was shaken at 30 °C for 1 h. The reaction was concentrated *in vacuo* to afford the final compounds as their TFA salts. LCMS QC: Column: Welch XB-C18 2.1x50mm 5 pm, 50 °C, mobile phase A: 0.0375% TFA in water; mobile phase B: 0.01875% TFA in acetonitrile. Initial gradient 15 1% B; 0.60 mins 5% B, 4.00 mins 100% B, 4.30 mins 1% B, 4.70 mins 1% B. Flow rate 0.8 mL/min. Preparative HPLC: Phenomenex Gemini C18; 250x21.2mmx10um; Acetonitrile:NH_4OH eluting with a gradient specific to each compound (see below) over an 8–10 min gradient time. Flow rate 30/35 mL/min unless otherwise specified.

4: Using *N*-boc-L-norvaline and an HPLC gradient of 59–89% organic. LCMS t_R = 2.33 min; MS *m/z* 246 $[M + H]^+$ HRMS for $C_{15}H_{24}N_3$ MS *m/z* $[M + H]^+$: Calc'd: 246.1960, Found: 246.1965.

6: Using *N*-boc-D-proline and an HPLC gradient of 54–84% organic. LCMS t_R = 2.40 min; MS *m/z* 244 $[M + H]^+$ HRMS for $C_{15}H_{21}N_3$ MS *m/z* $[M + H]^+$: Calc'd: 244.1808, Found: 244.1810.

7: Using *N*-boc-*trans*-4-hydroxy-L-proline and an HPLC gradient of 44–74% organic. LCMS t_R = 2.38 min; MS *m/z* 260 $[M + H]^+$

8: Using *N*-boc-D-asparagine and an HPLC gradient of 42–72% organic. LCMS t_R = 2.28 min; MS *m/z* 261 $[M + H]^+$ HRMS for $C_{14}H_{21}N_4O_1$ MS *m/z* $[M + H]^+$: Calc'd: 261.1710, Found: 261.1703.

14: Using *N*-boc-O-methyl-L-threonine and an HPLC gradient of 56–85% organic. LCMS t_R = 2.53 min; MS *m/z* 262 $[M + H]^+$ HRMS for $C_{15}H_{24}N_3O_1$ MS *m/z* $[M + H]^+$: Calc'd: 262.1914, Found: 262.1915.

6.4. 1-(6-(tert-Butyl)-1H-benzo[d]imidazol-2-yl)butan-1-ol (5)

The title compound was prepared in parallel format from 4-*tert*-butyl-1, 2-diaminobenzene and racemic 2-hydroxypentanoic acid using the following protocol. To 4-*tert*-butyl-1, 2-diaminobenzene (100 µmol, 0.333 M in dioxane, 1 eq) was added a solution of 2-hydroxypentanoic acid in dioxane (100 µmol, 1 eq) followed by diisopropylethyl amine (300 µmol, 3 eq) and a solution of T_3P in EtOAc (50% in EtOAc, 500 µL, 166.7 µmol, 1.67 eq). The reaction was shaken at 100 °C for 16 h before cooling and concentrating *in vacuo* to afford crude cyclized product which was purified by preparative HPLC: Preparative HPLC: Sepax BR-C18 column;100x21.2 mm x5µm; Acetonitrile:water (0.225% formic acid) eluting with 12–42% CH_3CN over 8 min gradient time. Flow rate 25 mL/min. LCMS QC: Column: Welch XB-C18 2.1x50mm 5 µm, 50 °C, mobile phase A: 0.0375% TFA in water; mobile phase B: 0.01875% TFA in acetonitrile. Initial gradient 1% B; 0.60 mins 5% B, 4.00 mins 100% B, 4.30 mins 1% B, 4.70 mins 1% B. Flow rate 0.8 mL/min. t_R = 2.369 min MS *m/z* 247 $[M + H]^+$

6.5. (2R,3S)-3-amino-3-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)-2-methylpropanamide (9)

tert-Butyl ((1S,2R)-3-amino-1-(5-(*tert*-butyl)-1H-benzo[*d*]imidazol-2-yl)-2-methyl-3-oxopropyl)carbamate (46) (286 mg, 0.765 mmol) was dissolved in dioxane (4 mL) and cooled in an ice bath. 4 M HCl in dioxane (4 mL), the ice bath removed and the reaction stirred for 3 h. The reaction was evaporated to dryness, water (4 mL) was added and then neutralized by adding solid $NaHCO_3$ (to pH = 7). The aqueous was extracted with 2-methylTHF (20 mL, then 3 × 10 mL). The combined organics were dried over Na_2SO_4, filtered and concentrated. The semi-solid residue was dissolved in acetonitrile (5 mL) and stirred at 50 °C. MeOH (0.5 mL) was added slowly, causing crystallization. After 12 h, the thick slurry was sonicated to loosen, then collected by filtration, washing with ice cold acetonitrile/MeOH (9/1) and dried at 40 °C under vacuum to afford 159 mg (76%) of (2R,3S)-3-amino-3-(5-(*tert*-butyl)-1H-benzo[*d*]imidazol-2-yl)-2-methylpropanamide (9) as a crystalline solid. ^1H NMR (400 MHz, CD_3OD) δ 7.54 (s, 1H), 7.46 (d, J = 8.40 Hz, 1H), 7.33 (dd, J = 1.76, 8.59 Hz, 1H), 4.18 (d,

J = 8.79 Hz, 1H), 2.84 (quin, J = 7.00 Hz, 1H), 1.37 (s, 9H), 1.03 (d, J = 7.03 Hz, 3H). MS m/z 275.24 $[M + H]^+$. HRMS for $C_{15}H_{23}N_4O_1$ MS m/z $[M + H]^+$ Calcd: 275.1866, Found: 275.1869.

6.6. (S)-3-(5-(tert-Butyl)-1H-benzo[d]imidazol-2-yl)-3-(methylamino) propanamide (10)

To a stirred solution of *tert*-butyl (S)-(3-amino-1-(5-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)-3-oxopropyl)(methyl)carbamate (57 mg, 0.15 mmol) in dioxane (1.20 mL) was added 4 M HCl in dioxane (1.14 mL, 4.56 mmol) dropwise. The reaction was stirred at room temperature for 2.5 hrs. The reaction was reduced in volume before loading (in MeOH) onto a SCX cartridge (2 g). Non-basic components were eluted using MeOH (pH ~ 5 after ~ 50 mL). 2 M NH_3 in MeOH was used to release product. The basic component was concentrated in vacuo to afford a white solid which was dried under vacuum. This solid was further purified by column chromatography (ISCO 4 g column, CH_2Cl_2/MeOH/NH_3 90:10:1) to isolate 32 mg (77%) of *(S)*-3-(5-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)-3-(methylamino)propanamide as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (br. s., 3H), 7.48 (br. s., 1H), 7.42 (s, 1H), 7.36 (br s, 1H), 7.19 (d, J = 8.20 Hz, 1H), 6.81 (br. s., 1H), 4.08 (dd, J = 6.25, 7.81 Hz, 1H), 2.51–2.64 (m, 2H), 2.16 (s, 3H), 1.31 (s, 9H). HRMS for $C_{15}H_{23}N_4O_1$ MS m/z $[M + H]^+$: Calc'd: 275.1866, Found: 275.1865.

6.7. (S)-3-Amino-3-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)-2,2-dimethylpropanamide (11)

tert-Butyl (S)-(3-amino-1-(5-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)-2,2-dimethyl-3-oxopropyl)carbamate (35 mg, 0.09 mmol) was dissolved in 1,4-dioxane (6 mL) and cooled to 0 °C. HCl (2 N in dioxane, 4 mL) was added and the reaction was stirred at room temp for 4 hr. After completion, the reaction was concentrated to obtained crude compound which was purified by ion exchange chromatography (SCX2 cartridge) to afford 25 mg (96%) of **11**. ^{1}H NMR (300 MHz, DMSO-d_6) δ11.9 (brs, 1H), 7.6–7.3 (m, 3H), 7.2 (d, J = *11 Hz,* 1H), 6.85 (brs, 1H), 4.24 (s, 1H), 2.2 (brs, 2H), 1.32 (s, 9H), 1.15 (s, 3H), 1.03 (s, 3H). HRMS for $C_{16}H_{25}N_4O_1$ MS m/z $[M + H]^+$: Calc'd: 290.2023, Found: 290.2025.

6.8. (1R,2R)-1-amino-1-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)propan-2-ol (12)

(R)-2-Amino-2-(5-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)ethan-1-ol (15)

(1R,2R)-1-amino-1-(6-isopropyl-1*H*-benzo[*d*]imidazol-2-yl)propan-2-ol (18)

(1R,2R)-1-amino-1-(5-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl) propan-2-ol (21)

The title compounds were prepared in parallel format using the following protocol: Step 1: To 4-alkyl-1, 2-diaminobenzene (100 μmol, 1 eq) was added a solution of the required amino acid in DMF (0.2 M, 500 μL, 100 μmol, 1 eq) followed by TEA (28 μL, 200 μmol, 2 eq) and a solution of HATU in DMF (0.2 M, 500 μL, 100 μmol, 1 eq). The reaction was shaken at 60 °C for 16 h before cooling and concentrating *in vacuo* to afford crude uncyclised intermediate. Step 2: To crude uncyclized intermediate was added acetic acid (1000 μL) and the reaction was shaken at 80 °C for 1 h. The reaction was cooled, concentrated *in vacuo* and dissolved in DMSO. The solution was filtered and purified using preparative HPLC to afford the intermediate benzimidazole. Step 3: To the intermediate benzimidazole was added CH_2Cl_2 (1800 μmol) followed by 4 M HCl in dioxane (200 μL) and the reaction was shaken at 30 °C for 1.5 h. The reaction was concentrated *in vacuo* to afford product as the HCl salt.

***(1R,2R)*-1-amino-1-(5-(*tert*-butyl)-1*H*-benzo[*d*] imidazol-2-yl) propan-2-ol (12)** (prepared from Boc-L-allo-threonine and 4-*tert*-butyl-1, 2-diaminobenzene) was purified by preparative HPLC: Preparative HPLC: Phenomenex Gemini C18; 250x21.2 mm x10μm; Acetonitrile:NH_4OH eluting with 37–67% CH_3CN over 10 min gradient time. Flow rate 30 mL/min. LCMS QC: Column: Welch XB-C18 2.1x50mm 5 μm, 50 °C, mobile phase A: 0.0375% TFA in water; mobile phase B: 0.01875% TFA in acetonitrile. Initial gradient 1% B; 0.60 mins 5% B, 4.00 mins 100% B, 4.30 mins 1% B, 4.70 mins 1% B. Flow rate 0.8 mL/min. t_R = 2.427 min. MS m/z 248 $[M + H]^+$ HRMS for $C_{14}H_{22}N_3O_1$ MS m/z $[M + H]^+$: Calc'd: 248.1757, Found: 248.1758.

***(R)*-2-Amino-2-(5-(*tert*-butyl)-1*H*-benzo[*d*] imidazol-2-yl)ethan-1-ol (15)** (prepared from *N*-Boc-L-serine and 4-*tert*-butyl-1, 2-diaminobenzene) was purified by preparative HPLC: Preparative HPLC: Phenomenex Gemini C18; 250x21.2 mm x10μm; Acetonitrile:NH_4OH eluting with 41–71% CH_3CN over 8.5 min gradient time. Flow rate 30 mL/min. LCMS QC: Column: Welch XB-C18 2.1x50mm 5 μm, 50 °C, mobile phase A: 0.0375% TFA in water; mobile phase B: 0.01875% TFA in acetonitrile. Initial gradient 1% B; 0.60 min 5% B, 4.00 min 100% B, 4.30 mins 1% B, 4.70 min 1% B. Flow rate 0.8 mL/min. t_R = 2.28 min MS m/z 234 $[M + H]^+$ HRMS for $C_{13}H_{19}N_3O_1$ MS m/z $[M + H]^+$: Calc'd: 234.1601, Found: 234.1604.

***(1R,2R)*-1-amino-1-(6-isopropyl-1*H*-benzo[*d*] imidazol-2-yl) propan-2-ol (18)** (prepared from Boc-L-allo-threonine and 4-isopropylbenzene-1,2-diamine) was purified by preparative HPLC: Preparative HPLC: Kromasil Eternity-5-C18 150x30mmx5μm; Acetonitrile-Water (0.225% formic acid) eluting with 23–53% MeCN over 10 min gradient time. Flow rate 30 mL/min. LCMS QC: Column: Welch XB-C18 2.1x50mm 5 μm, 50 °C, mobile phase A: 0.0375% TFA in water; mobile phase B: 0.01875% TFA in acetonitrile. Initial gradient 1% B; 0.60 min 5% B, 4.00 min 100% B, 4.30 min 1% B, 4.70 min 1% B. Flow rate 0.8 mL/min. t_R = 2.248 min. MS m/z 234 $[M + H]^+$

***(1R,2R)*-1-amino-1-(5-(trifluoromethyl)-1*H*-benzo[*d*] imidazol-2-yl)propan-2-ol (21)** (prepared from Boc-L-allo-threonine and 4-(trifluoromethyl)benzene-1,2-diamine) was purified by preparative HPLC: Preparative HPLC: Kromasil Eternity-5-C18 150x30mmx5μm; Acetonitrile-Water(0.225% formic acid) eluting with 23–53% MeCN over 10 min gradient time. Flow rate 30 mL/min. LCMS QC: Column: Welch XB-C18 2.1x50mm 5 μm, 50 °C, mobile phase A: 0.0375% TFA in water; mobile phase B: 0.01875% TFA in acetonitrile. Initial gradient 1% B; 0.60 min 5% B, 4.00 min 100% B, 4.30 min 1% B, 4.70 min 1% B. Flow rate 0.8 mL/min. t_R = 2.312 min. MS m/z 260 $[M + H]^+$ HRMS for $C_{11}H_{12}F_3N_3O_1$ MS m/z $[M + H]^+$: Calc'd: 260.1005, Found: 260.1006.

6.9. (R)-2-Amino-2-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)propan-1-ol (13)

To a solution of *(R)*-(2-(5-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)-1-hydroxypropan-2-yl)carbamate (92 mg, 0.27 mmol) in dioxane (3 mL) was added 4 M HCl in dioxane (4 mL) and the reaction stirred at room temperature for 3 h. The reaction was concentrated *in vacuo*, azeotroping with toluene (2 × 10 mL) 25 to afford a gummy solid. The residue was triturated with TBME (15 mL) to afford **13** as the bis HCl salt (55 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (br. s., 3H), 7.46–7.63 (m, 2H), 7.38 (dd, J = 1.56, 8.59 Hz, 1H), 3.78–3.92 (m, 2H), 1.68 (s, 3H), 1.33 (s, 9H). HRMS for $C_{14}H_{22}N_3O_1$ MS m/z $[M + H]^+$: Calc'd: 248.1757, Found: 248.1752.

6.10. (1R,2R)-1-amino-1-(5-(tert-butyl)-1H-benzo[d]imidazol-2-yl)-3-methylbutan-2-ol (16)

To the stirred solution of *tert*-butyl *(4R,5R)*-4-(5-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)-5-isopropyl-2,2-dimethyloxazolidine-3-carboxylate (60 mg.0.14 mmol) in dry dioxane (5 mL) was added 4 N HCl in dioxane (1.5 mL) at 0 °C then stirred at rt for 2 hr. The reaction mixture was concentrated under reduced pressure. The residue was triturated with ether twice to get 50 mg of crude product, which was passed through SCX2 cartridge (crude compound was dissolved in methanol

then loaded onto the SCX2 cartridge and methanol (5 mL) added. The desired compound eluted with methanolic ammonia). The methanolic ammonia solution was concentrated to afford 30 mg (75%) of **16**. ^{1}H NMR (300 MHz, DMSO-d_6): δ 12 (brs, 1H), 7.55–7.32 (m, 2H), 7.2 (d, J = 8 Hz, 1H), 4.75 (d, J = 6 Hz, 1H), 3.9 (d, J = 6 Hz, 1H), 3.5–3.42 (m, 1H), 2.2 (brs, 2H), 1.6–1.5 (m. 1H), 1.32 (s, 9H), 0.86 (d, J = 8 Hz, 6H). HRMS for $C_{16}H_{26}N_3O_1$ MS m/z $[M + H]^+$: Calc'd:276.2070, Found: 276.2079.

6.11. (1R,2R)-2-methoxy-1-(6-(trifluoromethoxy)-1H-benzo[d]imidazol-2-yl)propan-1-amine (17)

To a stirred solution of *tert*-butyl ((*1R,2R)*-2-methoxy-1-(6-(trifluoromethoxy)-1*H*-benzo[*d*]imidazol-2-yl)propyl)carbamate (1.63 g, 4.19 mmol) in dioxane (31 mL) was added 4 M HCl in dioxane (31 mL, 120 mmol). The solution turned dark blue/green in color and was stirred at room temperature for 2.5 h. The reaction was reduced in volume before loading (in MeOH) onto a SCX cartridge (10 g). MeOH washed through to remove non basic components (pH ~ 5 after ~ 150 mL). Basic eluent (2 M NH_3 in MeOH) then employed to release product. Basic component concentrated *in vacuo* to afford a red gummy solid which was left drying under vacuum for 4.5 days. This was purified using silica gel chromatography (80 g SiO_2, DCM/MeOH/NH_3 90:10:1). **17** was isolated as a white solid (1.15 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.55 (d, J = 8.59 Hz, 1H), 7.46 (br. s., 1H), 7.10 (d, J = 8.77 Hz, 1H), 3.99 (d, J = 4.69 Hz, 1H), 3.68 (dd, J = 4.88, 6.05 Hz, 1H), 3.31 (br. s., 2H), 3.21 (s, 3H), 1.06 (d, J = 6.25 Hz, 3H). HRMS for $C_{12}H_{15}F_3N_3O_1$ MS m/z $[M + H]^+$: Calc'd: 290.1111, Found: 290.1095.

6.12. 2-(2-((1R,2R)-1-Amino-2-hydroxypropyl)-1H-benzo[d]imidazol-5-yl)-2-methylpropanenitrile (19)

The title compound was prepared in parallel from 2-(3,4-diaminophenyl)-2-methylpropanenitrile and *N*-Boc-L-threonine using the following protocol: A 0.2 M solution of diamine in DMF (500 µL, 100 µmol) was added to a 0.2 M solution of acid in DMF (500 µL, 100 µmol) followed by HATU (100 µmol) and TEA (200 µmol). The reaction was stirred at 60 °C for 16 h. The reaction was concentrated *in vacuo* and AcOH (10 mL) was added to the residue. The reaction was stirred at 80 °C for 12 h, cooled and concentrated *in vacuo* to afford crude intermediate benzimidazole. To crude Intermediate benzimdazole was added CH_3CN (20 mL) followed by MSC Dowex resin (150 mg) and the reaction was stirred at 25 °C for 16 h. The resin was washed with 1:1 CH_3CN:MeOH followed by 5% NH_3/MeOH. The combined solvents were concentrated *in vacuo*, dissolved in DMSO (1 mL) and purified using preparative HPLC to afford **19**. LCMS QC Method:Column: RESTEK C18 2.1x30mm 3µ, mobile phase A: 0.05% formic acid in water; mobile phase B:Acetonitrile. Initial gradient 2% B; 0.75 min 2% B, 1.00 min 10% B, 2.00 min 98% B, 2.90 min 2% B, 3.00 min 2% B. Flow rate 1.5 mL/min. MS m/z $[M + H]^+$ 259. RT = 1.23 min. Preparative HPLC: Xterra 250x19mm, 10′ or X-Bridge 50x19mm, 5µ; mobile phase A: acetonitrile, mobile phase B: 0.05% NH3 in water; eluting with 5–35% CH_3CN over 7 min gradient time. Flow rate 20 mL/min. HRMS for $C_{14}H_1N_4O_1$ MS m/z $[M + H]^+$: Calc'd: 260.1582, Found: 260.1581.

6.13. (1R,2R)-1-amino-1-(6-(pentafluorosulfanyl)-1H-benzo[d]imidazol-2-yl)propan-2-ol (20).

4 M HCl in dioxane (10 mL) was added to a stirred, ice cooled solution of *tert*-butyl ((*1R,2R)*-2-hydroxy-1-(6-(pentafluorosulfanyl)-1*H*-benzo[*d*]imidazol-2-yl)propyl)carbamate (193 mg, 0.463 mmol) in dioxane and the reaction was stirred for 1hr at rt. The reaction mixture was concentrated under reduced pressure, and the resulting solid was triturated with EtOAc. The solid was dissolved in water, basified to pH 8 with a saturated solution of sodium hydroxide and extracted with EtOAc. The organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated to give 100 mg of a solid which was washed with 10% EtOH in pentane to afford 75 mg (51%) of **20**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10 (brs, 1H), 8.0 (m, 1H), 7.6 (m, 1H), 7.2 (m, 1H), 4.6 (m, 1H), 4.1 (m, 1H), 3.2 (brs, 1H), 1.2 (d, J = 9.8 Hz, 3H). HRMS for $C_{10}H_{12}F_5N_3O_1S_1$ MS m/z $[M + H]^+$: Calc'd: 318.0694, Found: 318.0692.

6.14. (R)-2,2-Dimethyl-4-(5-(1-methylcyclopropyl)-1H-benzo[d]imidazol-2-yl)oxazolidine (22)

tert-Butyl *(R)*-2,2-dimethyl-4-(5-(1-methylcyclopropyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-benzo[*d*]imidazol-2-yl)oxazolidine-3-carboxylate (200 mg, 0.240 mmol) was dissolved in 3 mL of CH_2Cl_2. Trifluoracetic acid (0.3 mL) was added and the reaction was stirred at room temperature for 6 h. An additional 1 mL of TFA was added and the reaction left to stir overnight. The solvent removed *in vacuo* and crude material was purified by chromatography (ISCO 12 g silica column, 100% CH_2Cl_2 to 80:20:2 CH_2Cl_2:MeOH:NH_4OH) to obtain 25 mg (45%) of **22** as a clear gum. ^{1}H NMR (400 MHz, CD_3OD) δ 7.46(m, 2H), 7.20(dd, J = 8.4, 1.6 Hz, 1H), 4.41(m, 1H), 3.93(m, 2H), 1.42(s, 3H), 0.86(m, 2H), 0.74(m, 2H). HRMS for $C_{13}H_{18}N_3O_1$ MS m/z $[M + H]^+$: Calc'd: 232.1444, Found: 232.1444.

6.15. (2R)-2-Amino-2-(5-(sec-butyl)-1H-benzo[d]imidazol-2-yl)ethan-1-ol (23)

tert-Butyl *(4R)*-4-(5-(*sec*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)-2,2-dimethyloxazolidine-3-carboxylate (76 mg, 0.20 mmol) was dissolved in 2 mL of 4 M HCl in dioxane and stirred at room temp overnight. The reaction was concentrated *in vacuo* to give a brown gum, which was purified by chromatography (SiO_2 column, 100% CH_2Cl_2 to 80:20:2 CH_2Cl_2:MeOH:NH_4OH) and concentrated *in vacuo* to give 30 mg (63%) **23** as a clear gum. ^{1}H NMR (400 MHz, CD_3OD) δ 7.44 (d, J = 8.20 Hz, 1H), 7.33 (s, 1H), 7.07 (dd, J = 1.46, 8.30 Hz, 1H), 4.14–4.23 (m, 1H), 3.85–3.95 (m, 1H), 3.76–3.85 (m, 1H), 2.69 (qd, J = 7.00, 14.13 Hz, 1H), 1.57–1.71 (m, 2H), 1.27 (d, J = 6.83 Hz, 3H), 0.80 (t, J = 7.42 Hz, 3H) HRMS for $C_{13}H_{20}N_3O_1$ MS m/z $[M + H]^+$: Calc'd: 234.1601, Found: 234.1602.

6.16. 4-(2-((1R,2R)-1-Amino-2-methoxypropyl)-1H-benzo[d]imidazol-5-yl)-3-fluorobenzonitrile (24)

tert-Butyl ((*1R,2R)*-1-(5-(4-cyano-2-fluorophenyl)-1*H*-benzo[*d*]imidazol-2-yl)-2-methoxypropyl)carbamate (95 mg, 0.022 mmol) was dissolved in a mixture of TFA and DCM (1 mL/5 mL). The reaction was stirred at room temperature for 3 h before concentrating *in vacuo*. The residue was purified by filtering through Amberlist-21 followed by reverse phase column chromatography eluting with 0–40% acetonitrile in 0.1% formic acid in water to afford the title compound as the formate salt (32 mg, 45%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.83 (br. s., 1H), 7.61–7.79 (m, 4H), 7.50 (d, J = 8.40 Hz, 1H), 4.30 (br. s., 1H), 3.87 (m, 1H), 3.43 (s, 3H), 1.17 (d, J = 6.25 Hz, 3H). HRMS for $C_{18}H_{18}F_1N_4O_1$ MS m/z $[M + H]^+$: Calc'd: 325.1459, Found: 325.1460.

6.17. 4-(2-((1S,2S)-1-amino-2-hydroxypropyl)-1H-benzo[d]imidazol-5-yl)benzonitrile (25)

4-(2-((*1S,2S)*-1-amino-2-hydroxypropyl)-1*H*-benzo[*d*]imidazol-5-

yl)benzonitrile was prepared from *N*-Boc-D-threonine in parallel using the following protocol: 3′,4′-Diamino-[1,1′-biphenyl]-4-carbonitrile (500 μL of a 0.25 M solution of in DMF, 125 μmole, 26.12 mg) was added to a reaction vial. To this was added 500 μL (125 μmole) of a 0.25 M solution of *N*-Boc-D-threonine in DMF followed by 125 μmole (47.5 mg) of HATU added as a neat solid. To this was added 125 μmole (18 μL) of triethylamine as neat liquid. The reaction was stirred at 60 °C for 16 hrs. The reaction mixture was evaporated in a Speedvac (2 hr, 5 torr, and 40 °C) and used directly in the second step cyclization without any further treatment. A 5:1 THF-AcOH (1 mL) was added to each of the reaction vials and the reactions were stirred at 60 °C for 16 hrs. Solvents were removed in a Speedvac (2 hr, 5 torr, and 50 °C). the crude reactions were dissolved in a minimum amount (500 μL) of CH_2Cl_2 and purified over silica gel (20% acetone in hexane). The purified products were taken up in CH_2Cl_2 (800 ul) in reaction vials and the vials cooled (5–10 °C). Trifluoroacetic acid (TFA) (200 μL) was added to each reaction vial and the reactions were stirred at 25 °C for 16 hrs. The TFA-CH_2Cl_2mixture was evaporated completely under reduced pressure in a Speedvac for 1 hr, (40 torr, 40 °C) and then for another 1 hr (at 5 torr, 40 °C). 1 mL of 20% Triethyl amine-DMF solution was added to each reaction vials and submitted for prep-HPLC purification. Preparative HPLC: XBRIDGE C18, 250 X 19 mm, 5um; Acetonitrile-water (0.1% NH_3) eluting with a gradient 10% - 50% acetonitrile over 23 min with a flow rate of 16 mL/min. QC: XBRIDGE C18 50 X 2.1 mm 2.5 um, 10 mM Ammonium acetate in water-acetonitrile, eluting with a gradient of 2% to 98% acetonitrile over 2 min with a flow rate of 1.5 mL/min. LCMS t_R = 1.3 min; MS *m/z* 292 $[M + H]^+$

6.18. 5-(2-((1R,2R)-1-amino-2-methoxypropyl)-1H-benzo[d]imidazol-5-yl)picolinonitrile (26)

tert-butyl ((*1R,2R*)-1-(5-(6-cyanopyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-yl)-2-methoxypropyl)carbamate (300 mg, 0.736 mmol) was dissolved in 4 mL of 4 M HCl in dioxane and stirred for 1 h at room temp. The reaction was quenched by the slow addition of saturated $NaHCO_3$ solution (20 mL). The reaction was extracted with EtOAc (3x20 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated. The crude material was purified by reverse phase chromatography to afford 110 mg (49%) of the title compound as the formate salt. ^{1}H NMR (400 MHz, CD_3OD) δ 9.04 (dd, J = 0.78, 2.34 Hz, 1H), 8.42 (s, 1H, formate), 8.28 (dd, J = 2.34, 7.81 Hz, 1H), 7.95 (d, J = 0.78 Hz, 1H), 7.93 (d, J = 0.78 Hz, 1H), 7.74 (d, J = 8.20 Hz, 1H), 7.60–7.69 (m, 1H), 4.27 (d, J = 6.64 Hz, 1H), 3.83 (quin, J = 6.35 Hz, 1H), 3.41 (s, 3H), 1.17 (d, J = 6.25 Hz, 2H). HRMS for $C_{17}H_{17}N_5O_1$ MS *m/z* $[M + H]^+$: Calc'd: 308.1506, Found: 308.1501.

6.19. (R)-2-amino-2-(7-(tert-butyl)quinoxalin-2-yl)ethan-1-ol (27)

Cold conc Hydrochloric acid (3 mL) was added to *tert*-butyl (R)-(2-(benzyloxy)-1-(7-(*tert*-butyl)quinoxalin-2-yl)ethyl)carbamate (120 mg, 0.27 mmol) at 0 °C then the reaction mixture was stirred at room temp for 14 h. The reaction mixture was concentrated then washed with ethyl acetate followed by diethyl ether and dried under vacuum to get 58 mg (75%) of the HCl salt of **27**. ^{1}H NMR (400 MHz, CD_3OD) δ 9.0 (s, 1H), 8.1 (s, 1H), 8.05–8.0 (m, 2H), 5–4.92 (m, 1H), 4.2–4.1 (m, 1H), 4.1–4.0 (m, 1H), 1.42 (s, 9H). MS *m/z* = 246.4 $[M + H]^+$

6.20. (S)-2-amino-2-(7-(tert-butyl)quinoxalin-2-yl)ethan-1-ol (28)

Prepared in the method of **27** to provide 55 mg (77%) of the HCl salt of **28**. ^{1}H NMR (400 MHz, CD_3OD) δ 9.0 (s, 1H), 8.1 (s, 1H), 8.05–8.0 (m, 2H), 4.2–4.1 (m, 1H), 4.1–4.0 (m, 1H), 1.42 (s, 9H). MS *m/z* = 246.2 $[M + H]^+$.

6.21. (R)-2-Amino-2-(6-(tert-butyl)quinoxalin-2-yl)ethan-1-ol (29)

Prepared in the method of **27** to provide 14 mg (73%) of the HCl salt of **29**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.98 (s, 1H), 8.14–8.01 (m, 3H), 4.15 (dd, J = *15, 7 Hz*, 1H), 4.05 (dd, J = *15, 9 Hz*, 1H), 1.42 (s, 9H). HRMS for $C_{14}H_{20}N_3O_1$ MS *m/z* $[M + H]^+$: Calc'd: 246.1601, Found: 246.1601.

6.22. (S)-2-Amino-2-(6-(tert-butyl)quinoxalin-2-yl)ethan-1-ol (30)

Prepared in the method of **27** to provide 3 mg (78%) of the HCl salt of **30**. ^{1}H NMR (400 MHz, CD_3OD) δ 8.98 (s, 1H), 8.14–8.01 (m, 3H), 4.15 (dd, J = *15, 7 Hz*, 1H), 4.05 (dd, J = *15, 9 Hz*, 1H), 1.42 (s, 9H). HRMS for $C_{14}H_{20}N_3O_1$ MS *m/z* $[M + H]^+$: Calc'd: 246.1601, Found: 246.1598.

6.23. (2R,3S)-3-Amino-3-(6-(tert-butyl)imidazo[1,2-a]pyridin-2-yl)-2-methylpropanamide (46)

HCl (0.28 mL, 5 N in iPrOH, 1.4 mmol) was added to the solution of a mixture of anti and syn amides (52 mg, 0.136 mmol) in MeOH (0.9 mL). The mixture was stirred at rt for 40 min. The solvent was removed and co-evaporated with CH_2Cl_2 twice, leading to a white solid. Diastereomers were separated using a Chiralpak AS-H column (10 × 250 mm, 80/20 CO_2/EtOH, 0.2% isopropylamine, 210 nM detection, later eluting compound was desired anti isomer)

46 ^{1}H NMR (400 MHz, CD_3OD): δ ppm 8.29, (s, 1H), 7.78 (s, 1H), 7.48 (m, 2H), 4.24, (d, J = *8.1 Hz*, 1H), 2.92 (quin, J = *8.0 Hz*, 1H), 1.39 (s, 9H), 1.09 (d, J = *8.0 Hz*, 3H). MS *m/z* 275 $[M + H]^+$ HRMS for $C_{15}H_{23}N_4O$ calcd: 275.1866; Found: 275.1865

syn isomer, (2S, 3S)-3-amino-3-(6-(*tert*-butyl)imidazo[1,2-*a*]pyridin-2-yl)-2-methylpropanamide ^{1}H NMR (500 MHz, CD_3OD) δ 8.84 (s, 1H), 8.42 (s, 1H), 8.23 (dd, J = *9.51, 0.98 Hz*, 1H), 7.93 (d, J = *9.51 Hz*, 1H), 4.91 (d, J = *7.07 Hz*, 1H), 3.34–3.42 (m, 1H), 1.45 (s, 9H), 1.40 (d, J = *7.07*, 3H). MS *m/z* 275 $[M + H]^+$. $[\alpha]_D^{23}$ = -0.134° (c = 1.0, MeOH)

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmc.2018.12.002.

References

1. Lampert AE, Mirjam Waxman, Stephen G. Altered sodium channel gating as molecular basis for pain: contribution of activation, inactivation, and resurgent currents. *Handbook Exp Pharmacol.* 2014;221:91–110.
2. Dib-Hajj SD, Cummins TR, Black JA, Waxman SG. Sodium channels in normal and pathological pain. *Annu Rev Neurosci.* 2010;33:325–347.
3. (a) Shields SD, Ahn H-S, Yang Y, et al. Nav1.8 expression is not restricted to nociceptors in mouse peripheral nervous system. Pain 2012:53:2017–2030.
(b) Faber CG, Lauria G, Merkies ISJ, et al. Gain-of-function Nav1.8 mutations in painful neuropathy. Proceedings of the National Academy of Sciences of the United States of America 2012, 109 (47), pp. 19444–19449, S19444/1-S19444/3.
(c) Fertleman CR, Baker MD, Parker KA, Moffatt S, Elmslie FV, Abrahamsen B, Ostman J, Klugbauer N, Wood JN, Gardiner RM, Rees M. SCN9A mutations in paroxysmal clinical study extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. *Neuron.* 2006;52(5):767–774;
(d) Dib-Hajj SD, Fjell J, Cummins TR, Zheng Z, Fried K, LaMotte R, Black JA, Waxman SG. Plasticity of sodium channel expression in DRG neurons in the chronic constriction injury model of neuropathic pain. *Pain.* 1999;83:591–600
4. Jarvis MF, Honore P, Shieh C-C, et al. A-803467, a potent and selective Nav1.8 sodium channel blocker, attenuates neuropathic and inflammatory pain in the rat. *Proc Natl Acad Sci USA.* 2007;104(20):8520–8525.

5. (a) Bungay SK, Denton PJ, Gibson SM, et al. Discovery and Optimization of Selective Nav1.8 Modulator Series That Demonstrate Efficacy in Preclinical Models of Pain. *ACS Med Chem Lett.* 2015;6(6):650–654
(b) Gibson SK, Kemp KR, Poinsard MI, et al. Pyridine derivatives as voltage-gated sodium channel modulators and their preparation, pharmaceutical compositions and use in the treatment of pain. WO2008135826, 20080421, 2008.
6. Bagal SK, Kemp MI, Bungay PJ, et al. Discovery and optimisation of potent and highly subtype selective Nav1.8 inhibitors with reduced cardiovascular liabilities. *MedChemComm.* 2016;7(10):1925–1931.
7. Bagal SK, Brown AD, Kemp MI, et al. Benzimidazole and imidazopyridine derivatives as Nav1.8 inhibitors and their preparation and use in the treatment of pain. WO2013114250A1, 2013.
8. Higashibayashi S, Tohmiya H, Mori T, Hashimoto K, Nakata M. Synthesis of sulfinimines by direct condensation of sulfinamides with aldehydes using Cs2CO3 as an activating and dehydrating reagent. *Synlett.* 2004;3:457–460.
9. Finke PE, Shah SK, Fletcher DS, et al. Orally Active β-Lactam Inhibitors of Human Leukocyte Elastase. 3. Stereospecific Synthesis and Structure-Activity Relationships for 3,3-Dialkylazetidin-2-ones. *J Med Chem.* 1995;38(13):2449–2462.