CHEMMEDCHEM

CHEMISTRY ENABLING DRUG DISCOVERY

Accepted Article

Title: Discovery of N-Aryloxypropylbenzylamines as Voltage-gated Sodium Channel NaV1.2 subtype Selective Inhibitors

Authors: Phillip L. van der Peet, Saman Sandanayake, Bevyn Jarrott, and Spencer John Williams

To be cited as: *ChemMedChem* 10.1002/cmdc.201800781

Link to VoR: http://dx.doi.org/10.1002/cmdc.201800781

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chemmedchem.org

WILEY-VCH

FULL PAPER

Discovery of *N*-Aryloxypropylbenzylamines as Voltage-gated Sodium Channel $Na_V1.2$ subtype Selective Inhibitors

Phillip L. van der Peet,[a] Saman Sandanayake,[a] Bevyn Jarrott,*[b] Spencer J. Williams*[a]

[a] Dr P. van der Peet, Dr S. Sandanayake, Prof. S.J. Williams 0000-0001-6341-4364
School of Chemistry and Bio21 Institute of Molecular Science and Biotechnology
University of Melbourne
Parkville, Vic. 3010, Australia
E-mail: sjwill@unimelb.edu.au
[b] Prof. B. Jarrott
Florey Institute of Neuroscience & Mental Health
Parkville, Vic. 3010, Australia
E-mail: bevyn.jarrott@florey.edu.au

Supporting information for this article is given via a link at the end of the document.

Abstract: We previously reported that a lipophilic *N*-(4'-hydroxy-3',5'-di-*tert*-butylbenzyl) derivative (**1**) of the voltage-gated sodium channel blocker mexiletine, was a more potent sodium channel blocker *in vitro* and *in vivo*. We demonstrate that replacing the chiral methylethylene linker between the amine and di-*tert*-butyl-phenol with an achiral 1,3-propylene linker (to give (**2**)) maintains potency *in vitro*. We synthesised 25 analogues bearing the 1,3-propylene linker and found that minor structural changes resulted in pronounced changes in state dependence of blocking human Na_V 1.2 and 1.6 channels by high throughput patch-clamp analysis. Compared to mexiletine, compounds **1** and **2** are highly selective $Na_V1.2$ inhibitors and >500 times less potent in inhibiting $Na_V1.6$ channels. On the other hand, a derivative (**4**) bearing 2,6-dimethoxy groups in place of the 2,6-dimethyl groups found in mexiletine was the most potent inhibitor but is non-selective against both channels in the tonic, frequency-dependent and inactivated states. In a kindled mouse model of refractory epilepsy compound **2** inhibited seizures induced by 6 Hz 44 mA electrical stimulation with an IC_{50} value of 49.9 ± 1.6 mg/kg. As established sodium channel blockers do not suppress seizures in this mouse model, this indicates that **2** could be a promising candidate for treating pharmaco-resistant epilepsy.

Introduction

Ion channels are heteromeric proteins that form gated, water-filled pores that span lipid bilayer plasma membranes and subcellular organelles and allow rapid movement of charged ions into and out of excitable cells in response to chemical stimuli, temperature changes or mechanical forces.[1] They are a large family of proteins with more than 300 genes encoding the subunits that confer different biochemical, biophysical and pharmacological properties, such as ion passage selectivity and inhibitor selectivity.[2] The proteins are dynamic and can adopt different conformations depending on membrane potential enabling the control of ion movement across the membrane. In mammals, one important class of ion channels are the voltage-gated sodium channels (Na_V), which are responsible for the initiation of electrical signalling in nerves and muscles. Nine isoforms of Na_V have been identified in humans; these are designated Na_V 1.1 to Na_V 1.9 and they have a high degree of sequence homology.[3] Furthermore, over 1,000 mutations have been identified in human Na_V channels that can lead to disorders such as epilepsy, neuropathic pain, arrhythmias, migraine, hyperthermia and other syndromes.[3] Blockade of Na_v by low molecular weight organic compounds has been used to manage but not cure these clinical conditions by inhibiting pathological firing patterns of neurons.

Voltage-gated sodium channels can exist in three main states: deactivated (closed), activated (open), or inactivated (closed).[4-5] In normal function, prior to an action potential, the axonal membrane is at its normal resting potential with sodium channels in the deactivated state. In response to membrane depolarization, the channel is activated, allowing sodium ions to flow into the neuron and causing further depolarization giving rise to action potential. At the peak of the action potential, the channel is inactivated and closes, stopping conduction of sodium ions and allowing repolarization of the axon. As the membrane potential falls, the channel returns to the deactivated state, ready to participate in another action potential.

Axons express two subtypes of sodium channels: $Na_V1.2$ and $Na_V1.6$ and these have different localizations. $Na_V1.6$ channels cluster in the nodes of Ranvier, where they trigger saltatory conduction and also in the distal end of the axon initial segment, where they promote action potential initiation along the axon.[6] Conversely, $Na_V1.2$ channels are densely localized in the proximal axon initial segment, where they promote back propagation to the soma and dendrites,[7] with preliminary medicinal chemistry leading to the development of $Na_V1.2$-selective drugs.[8] Additionally $Na_V1.6$ channels are present in microglia adjacent to axons and can trigger release of inflammatory cytokines in multiple sclerosis. Thus drugs blocking these subtypes could be neuroprotective against axonal damage. For example, CFM6058 is a selective inhibitor of $Na_V1.6$ and provides potent neuroprotective activity in hippocampal slices (Figure 1).[9]

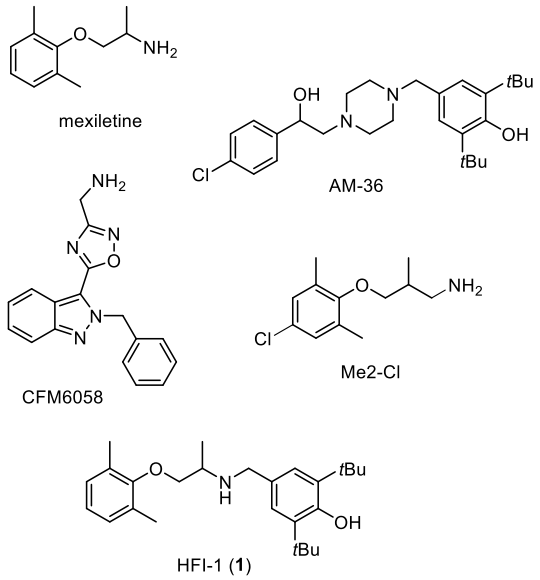


Figure 1. Selected sodium channel blockers.

Mexiletine was originally developed as a class 1B antiarrhythmic drug for the control of ventricular arrhythmias,[10-11] and is a frontline drug for the treatment of myotonic syndromes and possesses activity on the product of the *SCN4A* gene, $Na_V1.4$.[12] Early infantile epileptic encephalography has been found to be due to a mutation in the *SCN2A* gene expressing $Na_V1.2$ that causes a gain of function in the brain leading to tonic-clonic partial seizures and oral mexiletine has been found to reverse the epileptogenic dysfunction in these infants.[13] Also, it has been shown to relieve allodynia and hyperalgesia in rodent models of neuropathic pain, and affords relief in patients with neuropathic pain at relatively high doses (150-900 mg/d).[14] However, patient acceptance is poor with gastrointestinal nausea reported by 40% of patients.

Mexiletine represents an interesting lead compound for development of new Na_V blockers as it has high potency, yet has a low molecular weight, allowing for room to modify its structure without exceeding MW guidelines for drug-like compounds. Previous studies have reported variations in the aryloxy group and the linker of mexiletine. Carocci and co-workers showed that *meta*-hydroxymexiletine was equipotent on skeletal Na_V channels, and possessed superior potency on cardiac Na_V channels, and Desaphy and co-workers showed that hydroxylation of the aryloxy ring led to variations in potency of the resultant drugs that correlated with lipophilicity.[15] Conte-Camerino and co-workers showed that variations in the methylethylene group, by replacement of the methyl group with lipophilic substituents, led to derivatives more potent than mexiletine in use- and voltage-dependent block in frog muscle fibers.[16] Relevant to this work, the 2-methylpropyleneamine derivative of mexiletine exhibited similar potency to mexiletine in use-dependent block, while introduction of a 4-chloro group in Me2-Cl provided enhanced tonic and use-dependent block.[17]

Mexiletine has a low partition coefficient (logD = 0.39 at pH 7.4), which likely restricts its passage into the central nervous system. An analogue of mexiletine, HFI-1 (**1**), was developed with greater lipophilicity (logD = 5.53 at pH 7.4).[18] *In vitro*, HFI-1 was 82-fold more potent than mexiletine in displacing the radioligand [^{3}H]-batrachotoxinin in binding to Na^+ channels in depolarised rat brain membranes, and *in vivo*, was more effective than mexiletine as a drug candidate in reducing pain behaviors in three different rat models of neuropathic pain (formalin paw model, ligated spinal nerve model, and contusive spinal cord injury model).[18] HFI-1 shares the 3,5-*tert*-butyl-4-hydroxyphenylmethyl substituent with AM-36, a neuroprotective agent that has combined antioxidant and Na^+ channel blocking activity.[19]

In view of the potency of **1** as a Na_V ligand and a drug candidate we synthesized analogues of **1** designed to simplify the branched (and chiral) 2-aminopropyloxy linker within the molecule and determined the subsequent structure-activity relationships. We assessed the potency of these analogues for displacing [^{3}H]-batrachotoxinin from Na^+ channels in rat brain membranes, and then assessed the ability for these molecules to influence the electrophysiological properties of human $Na_V1.2$ and $Na_V1.6$ sodium channels expressed in Chinese hamster ovary cells. The most promising compound was evaluated in a mouse model of pharmacoresistant epilepsy.

Results and Discussion

Chemistry: Design and Synthesis

Compound **1** was designed to increase the lipophilicity of mexiletine to allow analogues to gain access to sodium channels within the central nervous system.[18] This was achieved by appending the antioxidant di-*tert*-butylphenol group, to allow inclusion of an antioxidant fragment that has previously been shown with AM-36 to reduce oxidative stresses within the CNS.[20-21] In developing a series of analogues of **1** we initially aimed to simplify the structure by replacing the branched 2-aminopropyloxy linker, which contains a stereogenic centre, with a linear 1,3-propylene linker, thereby avoiding issues with chirality. We initially prepared compound **2** by a three-step approach involving alkylation of 2,6-dimethylphenol with 3-bromopropylphthalimide, followed by removal of the phthalimide group by hydrazinolysis, and finally reductive amination with 3,5-di-*tert*-butylbenzaldehyde (Figure 2a). When assessed for binding to rat brain by displacement of the radioligand [^{3}H]-batrachotoxinin, a similar IC_{50} value was obtained relative to **1** (Table 1, *vide infra*). This promising result encouraged the preparation of additional derivatives maintaining the structure of the 3-aminopropyloxy linker and exploring variation in the nature of the left hand side aryloxy group, and the right hand side phenolic group.

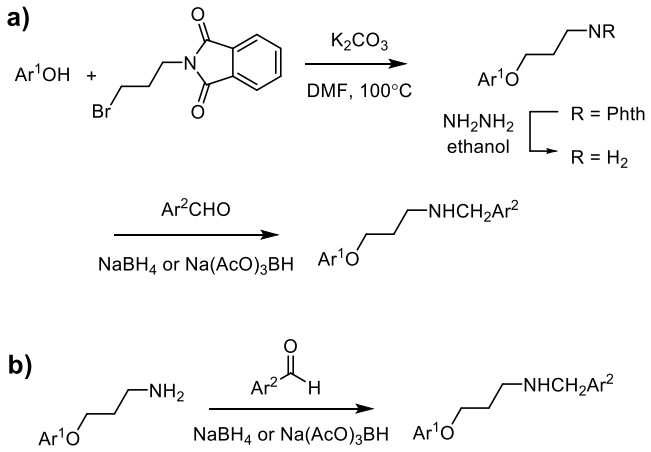


Figure 2. Synthesis of aryloxypropylbenzylamine derivatives.

Analogues that varied in the structure of the left-hand aryloxy group (Ar^1) were readily prepared from various commercially available phenols using the route in Figure 2a, and were chosen to explore variation in the size and nature of the 2,6-substituents, as well as the effect of introducing additional substituents (methyl, isopropyl, phenyl, thiophenyl) or nitrogen into the ring. Alternatively, the 2,6-dimethylphenyl ring was replaced by a pyrazole heterocycle. We also investigated analogues that varied the nature of the right-hand group (Ar^2) whilst maintaining the 2,6-dimethylphenyl group. These compounds were prepared from the aryloxypropylamine by reductive amination (Figure 2b).

Binding to the batrachotoxinin site in rat brain membranes

Voltage-sensitive sodium channels have a range of receptor sites for neurotoxins. Batrachotoxinin interacts with site 2, causing persistent activation of sodium channels, and is competitive with a range of effective sodium channel blocking drugs.[18] IC_{50} values for inhibition of binding of [^{3}H]-batrachotoxinin is shown for mexiletine and 14 analogues in Table 1. As we have previously reported, mexiletine competes with [^{3}H]-batrachotoxinin for binding to washed rat brain membranes with an apparent IC_{50} value of 11 μM, and appending the di-*tert*-butylphenoxy group to afford **1** results in an 82-fold enhancement of activity.[9] Conversion of the 2-methylethylene linker to the achiral propylene linker in **2** provided a small attenuation in activity relative to **1**, but was still 61-fold more potent than mexiletine. A range of variations to the 2,6-dimethylphenyl group were explored, including variation of the 2,6-dimethyl groups (**3-5**), conversion to a pyridine or pyrimidine heterocycle (**6**, **8**, **13**, **14**), and introduction of substituents at the 3 and 4 positions (**9,10,11**). In general, most variations led to either retention or reduction in activity. Notably, the 2,3,6-trimethylphenyl group (compound **12**) provided an approximately 2- and 3-fold enhancement in activity relative to compounds **1** and **2**, respectively.

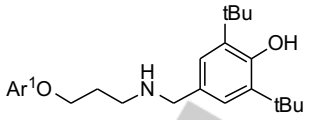
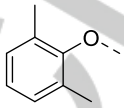
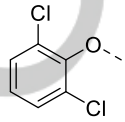
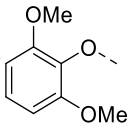
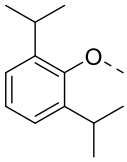
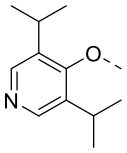
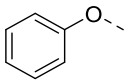
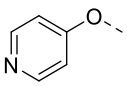
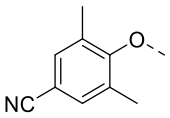
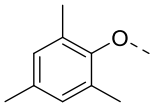
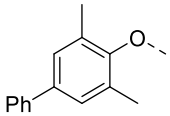
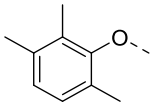
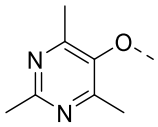
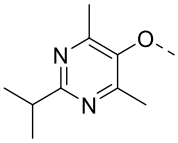


Table 1. IC_{50} values for Ar^1 variants binding to sodium channel site 2 in rat brain membranes.[a]

Compound	Ar^1O	IC_{50} (μM)
Mexiletine	-	11
1 (HFI-1)	-	0.13
2		0.18
3		0.10
4		0.11
5		0.09
6		0.38
7		0.24
8		0.87
9		0.15
10		0.28
11		0.12
12		0.056

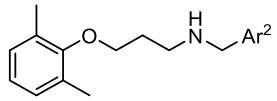
10.1002/cmdc.201800781

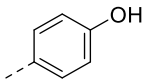
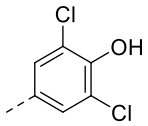
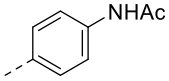
13		0.84
14		0.27

[a] IC_{50} values for binding to sodium channel site 2 in rat brain membranes determined in competition with [^{3}H]-batrachotoxinin.

Table 2 shows results for binding to sodium channel site 2 in rat brain membranes in competition with [^{3}H]-batrachotoxinin upon initial variation of the di-*tert*-butyl-4-hydroxyphenyl group. Removal of the *tert*-butyl groups to provide **15** gave a ~10-fold loss of activity relative to **1**, and replacement with chloro groups **16** led to loss of activity. Interestingly, the phenyl analogue **17** was only ~2-fold less potent than **2**; in this case conversion of the di-*tert*-butylphenoxy group of **2** to the phenyl of **17** results in a relatively large change in calculated LogD (pH 7.4): **2**, 3.7; **17**, 2.2. The acetamido derivative **18** was inactive.

Table 2. IC_{50} values for Ar^2 variants binding to sodium channel site 2 in rat brain membranes.[a]



Compound	Ar^2	IC_{50} (μM)
Mexiletine	-	11
1 (HFI-1)	-	0.13
15		1.19
16		>0.9
17		0.404
18		>0.9

[a] IC_{50} values for binding to sodium channel site 2 in rat brain membranes determined in competition with [^{3}H]-batrachotoxinin.

Electrophysiological assessment of binding to Nav 1.2 and 1.6

To gain greater insight into binding of the full suite of compounds **1**-**26** at specific channels important in the brain, we studied the state dependence of blocking of human $Na_V1.2$ and 1.6 channels using a high throughput patch-clamp system. Initial studies were performed using an Ionworks Quattro instrument. The voltage stimulus pattern is shown in Figure 3a. Test pulses applied at the indicated times allowed determination of tonic block (binding to resting closed channels); frequency-dependent block at a stimulation frequency of 10 Hz, and slow inactivated state block. All compounds were screened using protocol 1 as the primary screen. The results are shown in Tables 3 and 4. Initially, screening for compounds 1-8 were performed at a concentration range of 0.1–1000 μM; all other compounds were subsequently screened at a lower concentration range of 0.1–30 μM.

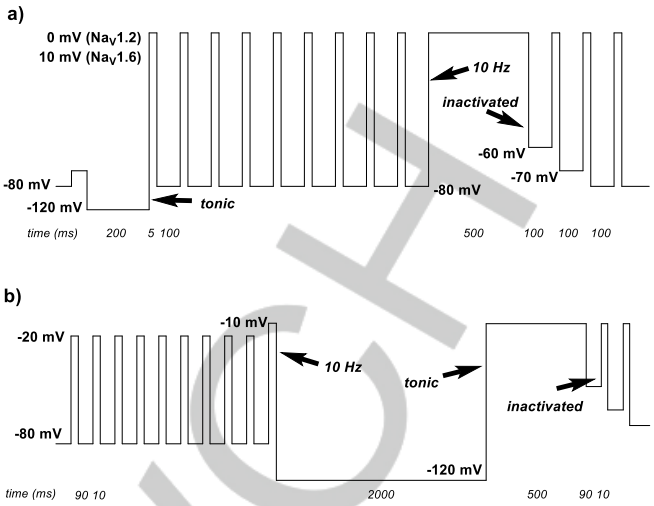
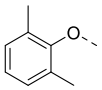
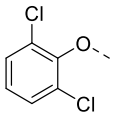
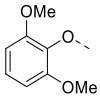
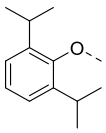
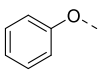
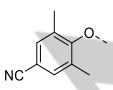
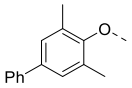
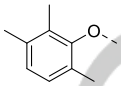
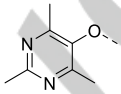
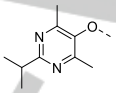
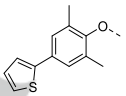
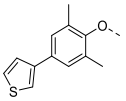
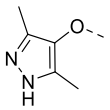
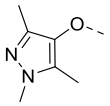
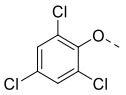


Figure 3. Patch clamp voltage protocols. a) Protocol 1 used for assessing state dependence of $Na_V1.2$ and 1.6 channels. The test-pulse potential was 0 mV for $Na_V1.2$; 10 mV for $Na_V1.6$. b) Protocol 2 allows assessment of contribution of binding to slow inactivated channels.

LHS structure-activity relationships: Inclusion of a phenyl or 2-thiophenyl group (compounds **11**, **19**) at the para position led to a loss of activity against $Na_V1.2$; compound **20**, which possesses a 3-thiophenyl group displayed some inhibition for the tonic and inactivated states. On the other hand, most other compounds displayed a marked enhancement in activity in binding to $Na_V1.2$ versus mexiletine for the tonic and 10 Hz states, and similar potencies for the inactivated states. Unlike mexiletine, which is a highly selective inhibitor of the inactivated state, most compounds displayed similar potencies across the three states. Interestingly, the trimethylpyrazole **22** is selective for the 10 Hz and inactivated states. For $Na_V1.6$, important differences are evident. Compounds **2**, **5**, **10**, **11**, **19**, **20**, and **23** did not significantly inhibit $Na_V1.6$. The structural factors responsible for lack of binding are complex, but it is clear that *para*-substituents are not tolerated. Compound **1** possessed similar activity towards $Na_V1.6$ as mexiletine, the remaining active compounds were much more potent than either compound, and mostly showed little selectivity across the three states. Notable exceptions include compounds **13** (>2-3-fold selectivity for 10 Hz and inactivated states) and **22** (9- and 16-fold selectivity for the 10 Hz and inactivated states). While mexiletine is 2-fold selective for $Na_V1.2$ versus $Na_V1.6$, many of the compounds display greatly altered selectivity. Notably, the data for compounds **1** and **2** reveal that changing the 2-methylethylene linker to the 1,3-propylene linker results in compounds with similar potency for $Na_V1.2$, but an enhancement in $Na_V1.2$:1.6 selectivity from approx. 1:80, to approx. 1:250 or better across all three states. For the remainder of the active compounds, selectivity for the two channels was more modest and usually in favour of $Na_V1.2$. However, compounds **8**, **13**, **21**, and **22** exhibited selectivites either close to unity or slightly in favour of $Na_V1.6$. The most potent compound of the series was compound **4**, which displayed sub-micromolar inhibition of all states of the two channels.

Table 3. Variation of Ar^1 on $Na_V1.2$ and $Na_V1.6$ channel affinities (Protocol 1).

	Ar^1	Block type	Nav1.2 IC_{50} (μM)	Nav1.6 IC_{50} (μM)	Nav1.2 selectivity [a]
Mexiletine	-	Tonic	122	256	2.1
		10 Hz	51.8	101	2.0
		Slow inactivated	8.2	23.5	2.9
1	-	Tonic	3.6	316	88
		10 Hz	3.9	324	83
		Slow inactivated	3.9	311	80
2		Tonic	4.1	>1000	>250
		10 Hz	3.3	>1000	>300
		Slow inactivated	2.4	>1000	>400
3		Tonic	2.0	6.0	3
		10 Hz	2.3	6.6	2.9
		Slow inactivated	2.1	5.3	2.5
4		Tonic	0.3	0.7	2.3
		10 Hz	0.4	0.8	2.0
		Slow inactivated	0.5	0.6	1.2
5		Tonic	6.3	>10	>1
		10 Hz	6.8	>10	>1
		Slow inactivated	7.4	>10	>1
7		Tonic	2.8	4.2	1.5
		10 Hz	2.5	3.5	1.4
		Slow inactivated	2.0	2.6	1.3
8		Tonic	>10	>10	-
		10 Hz	9.5	7.5	0.8
		Slow inactivated	5.7	5.9	1.0
9		Tonic	5.6	7.3	1.3
		10 Hz	4.2	6.2	1.5
		Slow inactivated	2.8	6.3	2.3
10		Tonic	3.1	>10	-
		10 Hz	3.5	>10	-
		Slow inactivated	3.9	>10	-
11		Tonic	>10	>10	-
		10 Hz	>10	>10	-
		Slow inactivated	>10	>10	-
12		Tonic	3.1	>30	>10
		10 Hz	3.5	>30	-
		Slow inactivated	3.8	>30	-
13		Tonic	>10	>10	-
		10 Hz	≈10.6	5.1	0.5
		Slow inactivated	5.0	3.5	0.7
14		Tonic	3.9	14.3	3.7
		10 Hz	2.4	12.8	5.3
		Slow inactivated	1.7	11.2	6.6
19		Tonic	>10	>10	-
		10 Hz	>10	>10	-
		Slow inactivated	>10	>10	-
20		Tonic	8.3	>10	-
		10 Hz	9.1	>10	-
		Slow inactivated	>10	>10	-
21		Tonic	9.0	4.8	0.5
		10 Hz	2.9	2.0	0.7
		Slow inactivated	2.3	1.3	0.6
22		Tonic	>30	29.0	-
		10 Hz	4.7	3.2	0.7
		Slow inactivated	3.2	1.8	0.6
23		Tonic	5.9	>10	-
		10 Hz	7.0	>10	-
		Slow inactivated	7.9	>10	-

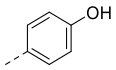
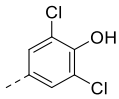
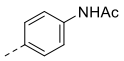
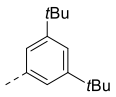
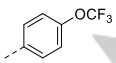
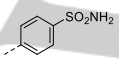
[a] In some cases the selectivity could not be calculated; an estimate is provided if >10.

RHS structure-activity relationships: Compound **15**, lacking the *t*-butyl groups present in compound **2**, maintained activity at $Na_V1.2$ for the 10 Hz and inactivated states, but possessed reduced activity at the tonic state. Interestingly, inhibitory activity

was gained for the other states for $Na_V1.2$, leading to similar potency for the two channels and no selectivity. Compound **16,** in which chloro groups replace the *t*-butyl groups in compound **2**, showed selectivity for the inactivated state of $Na_V1.2$, and no activity for $Na_V1.6$. Compound **24**, in which the phenolic group of **2** is absent, possessed no activity against either channel. Strikingly, the benzyl derivative **17** showed activity against all three states of the two Na_V channels, with submicromolar potency and some selectivity for the inactivated state of $Na_V1.2$.

Compounds **18** and **26**, which possess 4-acetamido or sulfonamido groups, displayed similar profiles of inhibition against only the inactivated states of $Na_V1.2$ and $Na_V1.6$; and similar selectivities weakly in favour of $Na_V1.2$. Finally, compound **25** bearing a 4-trifluoromethoxy group, showed a 10-fold or greater selectivity for various states of $Na_V1.2$, with a preference for binding to the inactivated state of the channels.

Table 4. Variation of Ar^2 on $Na_V1.2$ and $Na_V1.6$ channel affinities (Protocol 1).

	Ar^1	Block type	$Na_V1.2$ IC_{50} (μM)	$Na_V1.6$ IC_{50} (μM)	$Na_V1.6$/ selectivity [a]
15		Tonic	>10	>10	-
		10 Hz	5.1	4.8	0.9
		Slow inactivated	2.2	2.2	1.0
16		Tonic	>10	>10	-
		10 Hz	>10	>10	-
		Slow inactivated	4.4	>10	-
17		Tonic	3.3	5.2	1.6
		10 Hz	1.8	2.9	1.6
		Slow inactivated	0.5	2.0	4.0
18		Tonic	>10	>10	-
		10 Hz	>10	>10	-
		Slow inactivated	5.0	7.1	1.4
24		Tonic	>10	>10	-
		10 Hz	>10	>10	-
		Slow inactivated	>10	>10	-
25		Tonic	12.5	137.8	11
		10 Hz	3.6	56.3	16
		Slow inactivated	1.4	40.9	29
26		Tonic	>10	>10	-
		10 Hz	>10	>10	-
		Slow inactivated	6.1	8.5	1.4

[a] In some cases the selectivity could not be calculated.

Among the set of compounds studied, compounds **1** and **2** were selected on the basis of high $Na_V1.2$ selectivity and high lipophilicity for additional studies. In these studies we sought to confirm binding using an alternative patch clamp protocol, and also to examine the potential for slow binding. Protocol 2 was performed on an IonWorks Barracuda instrument, following the method of Kirsch (Figure 3b).[22] This protocol combines a 10 Hz frequency-dependent voltage sequence with a long (2s) delay with peak currents measured at -10 mV, and will enhance the contribution of slow inactivated channels to the measured block. To minimize the potential for variation between individual assays, compound inhibition of $Na_V1.2$ and $Na_V1.6$ activities were assessed on the same plate at the same time. Also included in this analysis were positive controls, lamotrigine and lidocaine, which are reported to modulate Na_V channel fast inactivation. Interestingly, compounds **1** and **2** both display reasonable potencies for the inactivated state of $Na_V1.6$. Collectively, these data suggest that compounds **1** and **2** exhibit fast binding kinetics to the inactivated state of $Na_V1.2$, and slow binding kinetics for the inactivated state of $Na_V1.6$.

Table 5. Affinities for binding to $Na_V1.2$ and $Na_V1.6$ channels primarily in the slow inactivated state (Protocol 2).

	Type of inhibition	$Na_V1.2$ IC_{50} (μM)	$Na_V1.6$ IC_{50} (μM)	$Na_V1.2$ selectivity[a]
1	Tonic	18	>30	-
	10 Hz	13	>30	-
	Slow inactivated	5.1	23	4.6
2	Tonic	18.8	>30	-
	10 Hz	15.3	>30	-
	Slow inactivated	5.6	18	3.2
lamotrigine	Tonic	>300	>300	-
	10 Hz	>300	>300	-
	Slow inactivated	94	50	0.5
lidocaine	Tonic	2290	2150	~1
	10 Hz	680	565	0.8
	Slow inactivated	50	75	0.7

[a] In some cases the selectivity could not be calculated.

Metabolic stability and anti-epileptic testing of compound 2

The metabolic stability of compounds 1 and 2 were assessed in human liver microsomes in the presence of NADPH. According to the data in Table 6, the compounds undergo high and intermediate clearance levels. Preliminary identification of metabolites by mass spectrometry revealed compound 1 undergoes *O*-dealkylation to the alcohol, *N*-dealkylation to mexiletine, and mono and bis-oxygenation. Compound 2 underwent equivalent *N*-dealkylation, and monooxygenation.

Table 6. Metabolic evaluation of compounds **1** and **2** in human liver microsomes.[a]

Cmpd	$T_{1/2}$ (min)	$CL_{int,\ in\ vitro}$ (µL/min/mg protein)	Predicted $CL_{int,\ in\ vivo}$ (mL/min/kg)	Predicted CL_{blood} (mL/min/kg)	Predicted E_H
1[b]	11	162	133	18	0.87
2	62	28	23	11	0.52

[a] Assays were performed with an NADPH regenerating system.
[b] Apparent minor non-NADPH mediated degradation was observed in metabolism control samples (20 - 30% degradation over 60 min), however no putative metabolites were detected. Predicted in vivo clearance parameters are reported; however, these may be an underestimation of the true values.

On the basis of its better stability relative to compound **1**, compound **2** was tested for antiepileptic activity *in vivo* in mice by the Epilepsy Therapy Screening Program (ETSP) carried out by the National Institute of Neurological Disorders and Stroke (Bethesda, MD). Its current strategy has progressed beyond using acute anticonvulsant screens such as the maximal electroshock seizure and pentylenetetrazole tests to a mouse screen of pharmaco-resistant epilepsy.[23] This is a low frequency (6 Hz) electrical stimulation of brain at 22 mA for 3 seconds that induces seizures reminiscent of 'psychomotor seizures' seen in human limbic epilepsy. By increasing the current to 44 mA, the conventional Na_V blockers phenytoin and lamotrigine do not block seizures so this is used as a model of therapy-resistant limbic seizures.[24-25] Interestingly, intraperitoneal injection of compound **2** blocked these seizures and had a calculated ED_{50} of 49.9 ± 1.65 mg/kg. Separate groups of mice were injected with higher doses of **2** and 6 hours later they were tested on a rota-rod apparatus to measure any motor impairment. The dose causing 50% of mice to fall off the rotating rod was calculated to be 90.5 ± 2.7 mg/kg. Thus **2** is a potential candidate for treating pharmaco-resistant epilepsy.

Conclusions

Here we report an examination of structure-activity relationships for mexiletine against two neurologically significant sodium channels, $Na_V1.2$ and $Na_V1.6$. Alkylation of the amine nitrogen enhanced lipophilicity and dramatically enhanced potency for $Na_V1.2$ versus $Na_V1.6$. Furthermore, conversion of the chiral methylethylene linker to an achiral propylene linker did not significantly affect potency. Further, modifications to the 2,6-dimethylphenyl group resulted in a wide range of selectivities for $Na_V1.2$ versus $Na_V1.6$, and a spectrum of activities across the various states of each channel. While inhibition of the inactivated state is common for existing Na_V-blocking drugs, and is thought to improve the safety margin by targeting the pathological state in conditions such as epilepsy[26] and ischemia, new Na_V-blocking drugs targeting other disorders may be of utility in treating conditions such as multiple sclerosis where demyelination of CNS axons is present and amyotropic lateral sclerosis where Na^+-mediated hyperexcitability is involved.[26] Additionally, the low lipophilicity of mexiletine results in its exclusion from the CNS and consequent activity only on peripheral Na_V channels. Our work reveals more lipophilic variants that have the potential to cross the blood-brain barrier and modulate Na_V channels in the CNS. Preliminary *in vivo* studies with **2** indicated that it provides antiseizure actions in a mouse model of pharmaco-resistant epilepsy in contrast to established sodium channel blockers such as phenytoin and lamotrigine.[24] Compound **2** would be a worthwhile candidate for detailed pharmacodynamic and pharmacokinetic studies.

Experimental Section

The preparation of compounds HFI-1 (**1**), and **2**-**8** have been reported previously.[27]

General methods for reductive amination

Method A1

A mixture of amine (1 eq.), aldehyde (1.2 eq.) and MeOH (5 mL/mmol amine) was stirred at room temperature under N_2 until no more amine was visible by thin-layer chromatography. $NaBH_4$ (1-2 eq.) was added and the mixture was stirred at r.t. for 30-60 min. The mixture was poured into sat. aq. $NaHCO_3$ and extracted with Et_2O or EtOAc (3 times). The combined organic extracts were dried ($MgSO_4$), filtered, and the crude mixture purified according to Protocol A or B (below) to obtain products as their pure hydrochloride salts (see below).

Method A2

Where imine formation did not occur at room temperature in MeOH according to Method A1 as assessed by thin-layer chromatography, the reaction was instead conducted in EtOH at reflux. Upon consumption of amine, the reaction mixture was cooled to r.t. prior to $NaBH_4$ addition as per Method A1.

Method B

A mixture of amine (1 eq.), aldehyde (1.2 eq.) and 1,2-dichloroethane (5-10 mL/mmol amine) was stirred at room temperature under N_2 for 1 h. $NaBH(OAc)_3$ (1.4 eq.) was added and the mixture was stirred for 2-4 h then poured into sat. aq. $NaHCO_3$and extracted with EtOAc. The organic phase was washed sequentially with sat. aq. $NaHCO_3$, H_2O, then brine, dried ($MgSO_4$), filtered, and the crude solution of product treated in one of two ways to obtain products as their pure hydrochloride salts (see below). Owing to the tendency for this reaction to give tertiary amine products as well as the desired hydrochloride salts, the crude material was preferably purified using Purification Method B.

Purification Protocols

Protocol A

For reactions where very little starting amine remained the organic extract was treated with 1 M HCl in MeOH affording a crystalline precipitate that was collected by vacuum filtration. In cases where crystals did not form, mixtures were evaporated and crystallized from either MeOH/Et_2O, MeOH/H_2O, EtOH/H_2O, EtOH/THF, MeCN/H_2O or MeOH/THF, as noted. If necessary compounds were purified by recrystallization using the aforementioned solvent mixtures.

Protocol B

The dried organic extract was concentrated in vacuo and pure free-base was purified by flash chromatography using either EtOAc/petroleum spirits (10→100% with 1-2% Et_3N) or EtOAc/MeOH/H_2O/Et_3N (97:2:1:2 → 7:2:1:0.2). After evaporation of the eluent the remaining Et_3N was removed by azeotroping with dichloromethane. The free base was converted to the hydrochloride salt by either dissolving in Et_2O and adding 1 M HCl in MeOH or dissolving in MeOH and adding aq. HCl (either 1 M or 10 M). Where necessary compounds were crystallized or recrystallized from MeOH/Et_2O, MeOH/H_2O, EtOH/H_2O, EtOH/THF, MeCN/H_2O or MeOH/THF, as noted.

Preparation of 4-(3-(3,5-di-*tert*-butyl-4-hydroxybenzylamino)propyloxy)-3,5-dimethylbenzonitrile (9).

i) 4-(3-Bromopropyloxy)-3,5-dimethylbenzonitrile (27). A mixture of 1,3-dibromopropane (683 mg, 3.39 mmol), 4-cyano-2,6-dimethylphenol (100 mg, 0.679 mmol), Cs_2CO_3 (331 mg, 1.02 mmol) and acetone (20 mL) was heated at reflux for 16 h. The mixture was filtered, concentrated under reduced pressure and the residue purified by flash chromatography (20% EtOAc/petroleum spirits) to afford the title compound (163 mg, 95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.30 (6 H, s, 2 × CH_3), 2.33 (2 H, quint, *J* 6.2 Hz, C**H**$_2$CH_2N), 3.69 (2 H, t, *J* 6.2 Hz, CH_2N), 3.93 (2 H, t, *J* 6.2 Hz, CH_2O), 7.33 (2 H, d, *J* 0.5 Hz, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=16.4, 30.1, 33.39, 69.55, 107.7, 119.2, 132.8, 132.9, 133.0, 159.6; HRMS-ESI *m/z* calcd. for [$C_{12}H_{14}$BrNO+H]$^+$: 268.0343, found: 268.0337.

ii) 4-(3-Azidopropyloxy)-3,5-dimethylbenzonitrile (28). A mixture of compound **27** (163 mg, 0.648 mmol), NaN_3 (46.3 mg, 0.711 mmol) and DMSO (2 mL) was stirred overnight at r.t. Water (20 mL) was added and the mixture was extracted with Et_2O (2 × 50 mL). The combined organic extracts were washed with H_2O (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the title compound (384 mg, 98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.07 (2 H, quint, *J* 6.2 Hz, C**H**$_2$CH_2N), 2.29 (6 H, s, 2 × CH_3), 3.61 (2 H, t, *J* 6.2 Hz, CH_2N), 3.87 (2 H, t, *J* 6.2 Hz, CH_2O), 7.38 (2 H, s, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ=16.3, 29.8, 48.4, 69.4, 107.7, 119.1, 132.7, 133.0, 159.6; HRMS-ESI *m/z* calcd. for [$C_{12}H_{14}N_4O$+H]$^+$: 231.1245, found: 231.1243.

iii) 4-(3-Aminopropyloxy)-3,5-dimethylbenzonitrile (29). A mixture of Ph_3P (200 mg, 0.763 mmol), compound **28** (384 mg, 0.630 mmol), water (2 mL) and THF (10 mL) was stirred at r.t. overnight. The mixture was poured into 10% aq. NaOH (30 mL) and extracted with EtOAc (2 × 20 mL). The combined organic extracts were dried ($MgSO_4$), filtered and concentrated under reduced pressure. Flash chromatography (17:2:1 EtOAc/MeOH/H_2O with 2% Et_3N) gave the title compound (285 mg, 88%).

iv) 4-(3-(3,5-Di-*tert*-butyl-4-hydroxybenzylamino)propyloxy)-3,5-dimethylbenzonitrile (9). Method A1 applied to compound **29** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde followed by purification Protocol B (MeOH/H_2O) afforded the title compound (74 mg, 34%). ^{1}H NMR (400 MHz, CD_3OD): δ=1.44 (18 H, s, 2 × C(CH_3)$_3$), 2.17-2.25 (2 H, m, C**H**$_2$CH_2N), 2.29 (6 H, s, 2 × CH_3), 3.27-3.35 (2 H, obscured t, CH_2N), 3.94 (2 H, t, *J* 6.0 Hz, CH_2O), 7.30 (2 H, s, Ar), 7.43 (2 H, s, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=16.3, 28.0, 30.6, 35.6, 45.8, 53.0, 70.4, 108.7, 119.7, 123.0, 128.0, 134.0, 134.1, 140.0, 156.6, 160.7; HRMS-ESI *m/z* calcd. for [$C_{27}H_{38}N_2O_2$+H]$^+$: 423.3011, found: 423.3008.

Preparation of 2,6-Di-*tert*-butyl-4-((3-(2,4,6-mesityloxy)propylamino)methyl)phenol (10).

i) *N*-(3-(2,4,6-Trimethylphenoxy)propyl)phthalimide (30). A mixture of 2,4,6-trimethylphenol (2.66 g, 19.5 mmol), *N*-(3-bromopropyl)phthalimide (5.00 g, 18.6 mmol), K_2CO_3 (2.70 g, 19.8 mmol) and DMF (20 mL) was heated at 110 °C for 3 h. The mixture was concentrated under reduced pressure then partitioned between H_2O and dichloromethane (50 mL of each). The organic layer was separated and washed sequentially with 0.3 M aq. NaOH (3 × 50 mL), H_2O (50 mL), then sat. aq. NaCl (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was crystallized from hot methanol (25 mL) to afford the title compound as crystals (2.90 g, 48%).

ii) 3-(2,4,6-Mesityloxy)propan-1-amine (31). A mixture of compound **30** (2.85 g, 8.65 mmol), hydrazine hydrate (3.53 g, 52 mmol) and ethanol (50 mL) was heated in an oil bath at 100 °C for 3 h under N_2. After cooling to r.t. the solvent was removed under reduced pressure and the residue treated with 25% aq. NaOH. The mixture was extracted with EtOAc (3 × 20 mL), the combined organic extracts were dried ($MgSO_4$), concentrated under reduced pressure and the residue purified by flash chromatography (17:2:1 EtOAc/MeOH/H_2O with 2 % Et_3N) to give the title compound (1.47 g, 86%). ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ=1.78 (2 H, quintet, *J* 6.6 Hz, C**H**$_2$CH_2N), 2.16 (6 H, s, 2 × CH_3), 2.17 (3 H, s, CH_3), 2.74 (2 H, t, *J* 6.8 Hz, CH_2N), 3.72 (2 H, t, *J* 6.4 Hz, CH_2O), 6.79 (2 H, s, Ar).

iii) 2,6-Di-*tert*-butyl-4-((3-(2,4,6-mesityloxy)propylamino)methyl)phenol (10). Method A1 applied to compound **31** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde followed by purification by Protocol B (MeOH/H_2O) afforded the title compound (494 mg, 71%). ^{1}H NMR (500 MHz, CD_3OD): δ=1.44 (18 H, s, 2 × C(CH_3)$_3$), 2.18 (11 H, m, 3 × CH_3,C**H**$_2$CH_2N), 3.32 (2 H, m, CH_2N), 3.83 (2 H, t, *J* 5.8 Hz, CH_2O), 4.17 (2 H, s, ArCH_2N), 6.79 (2 H, s, Ar), 7.33 (2 H, s, Ar); ^{13}C NMR (125 MHz, CD_3OD): δ=16.4, 20.8, 28.0, 30.6, 35.6, 46.1, 52.9, 70.2, 123.0, 128.0, 130.5, 131.2, 134.6, 140.0, 154.3, 156.6; HRMS-ESI *m/z* calcd. for [$C_{27}H_{41}NO_2$+H]$^+$: 412.3215, found: 412.3228.

Preparation of 2,6-di-*tert*-butyl-4-((3-(3,5-dimethylbiphenyl-4-yloxy)propylamino) methyl)phenol (11).

i) *N*-(3-(3,5-Dimethylbiphenyl-4-yloxy)propyl)phthalimide (32). A mixture of 4-phenyl-2,6-dimethylphenol (351 mg, 1.77 mmol), *N*-(3-bromopropyl)phthalimide (475 mg, 1.94 mmol), Cs_2CO_3 (1.15 g, 3.54 mmol) and acetone was heated at reflux for 16 h. The mixture was filtered and concentrated under reduced pressure to give a thick oil, which was purified by flash chromatography (10-15% EtOAc/petroleum spirits) gave the title compound (566 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.23 (2 H, quint, *J* 6.2 Hz, C**H**$_2$CH_2N), 2.33 (6 H, s, 2 × CH_3), 3.90, 3.98 (4 H, 2 × t, *J* 6.2 Hz, CH_2N,CH_2O), 7.22 (2 H, s, Ar), 7.27-7.32 (1 H, m, Ar), 7.37-7.43 (2 H, m, Ar), 7.51-7.55 (2 H, m, Ar), 7.70-7.75 (2 H, m, Ar), 7.82-7.88 (2 H, m, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=16.7, 29.6, 35.7, 70.0, 123.3, 126.9, 127.0, 127.7, 128.7, 131.2, 132.3, 134.0, 136.8, 141.0, 155.7, 168.4; HRMS-ESI *m/z* calcd. for [$C_{25}H_{23}NO_3$+H]$^+$: 386.1756, found: 386.1765.

ii) 3-(3,5-Dimethylbiphenyl-4-yloxy)propan-1-amine (33). A mixture of **32** (546 mg, 1.41 mmol), hydrazine hydrate (578 mg, 8.5 mmol) and ethanol (50 mL) was heated in an oil bath at 100 °C for 3 h under N_2. After cooling to r.t. the solvent was removed under reduced pressure and the residue treated with 25% aq. NaOH. The mixture was extracted with EtOAc (3 × 20 mL), the combined organic extracts were dried ($MgSO_4$), concentrated under reduced pressure and the residue purified by flash chromatography (17:2:1 EtOAc/MeOH/H_2O with 2 % Et_3N) to give the title compound (384 mg, 100%). ^{1}H NMR (400 MHz, CD_3OD): δ=1.98 (2 H, quint, *J* 6.2 Hz, C**H**$_2$CH_2N), 2.33 (6 H, s, 2 × CH_3), 2.92 (2 H, t, *J* 6.2 Hz, CH_2N), 3.87 (2 H, t, *J* 6.2 Hz, CH_2O), 7.23-7.28 (3 H, m, Ar), 7.34-7.40 (2 H, m, Ar), 7.49-7.54 (2 H, m, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=16.8, 34.5, 40.1, 71.6, 127.7, 127.8, 128.4, 129.6, 132.1, 138.0, 142.2, 156.7; HRMS-ESI *m/z* calcd. for [$C_{17}H_{21}NO$+H]$^+$: 256.1701, found: 256.1704.

iii) 2,6-Di-*tert*-butyl-4-((3-(3,5-dimethylbiphenyl-4-yloxy)propylamino)methyl)phenol (11). Method A1 applied to **33** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde followed by purification according to Protocol B (MeOH/H_2O) afforded the title compound (210 mg, 27%). ^{1}H NMR (500 MHz, CD_3OD): δ=1.45 (18 H, s, 2 × $C(CH_3)_3$), 2.22 (2 H, m, C**H**$_2$$CH_2N$), 2.30 (6 H, s, 2 × CH_3), 3.35 (2 H, m, CH_2N), 3.83 (2 H, t, *J* 5.8 Hz, CH_2O), 4.18 (2 H, s, $ArCH_2N$), 7.23-7.32 (5 H, m, Ar), 7.39 (2 H, t, *J* 7.6 Hz, Ar), 7.53 (2 H, t, *J* 7.8 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ=16.6, 28.1, 46.1, 53.0, 70.2, 123.0, 127.8, 128.0, 128.5, 129.7, 132.1, 138.5, 140.1, 142.1, 156.2, 156.6; HRMS-ESI *m/z* calcd. for $[C_{32}H_{43}NO_2+H]^+$: 474.3372, found: 474.3367.

Preparation of 2,6-di-*tert*-butyl-4-((3-(2,3,6-trimethylphenoxy)propylamino)methyl) phenol (12).

i) *N*-(3-(2,3,6-Trimethylphenoxy)propyl)phthalimide (34). A mixture of 2,3,6-trimethylphenol (990 mg, 7.27 mmol), *N*-(3-bromopropyl)phthalimide (2.14 g, 8.00 mmol), K_2CO_3 (3.01 g, 21.8 mmol) and DMF (5 mL) was heated at 70 °C for 2 h. The mixture was concentrated under reduced pressure then partitioned between EtOAc and sat. aq. $NaHCO_3$ (50 mL of each). The organic layer was separated and the aqueous layer extracted with EtOAc (2 × 20 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$ (50 mL) then sat. aq. NaCl (50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (10-15% EtOAc/petroleum spirits) to give the title compound (1.01 g, 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.17-2.24 (11 H, m, 3 × CH_3,C**H**$_2$$CH_2O$), 3.82 (2 H, t, *J* 6.3 Hz, CH_2N), 3.96 (2 H, t, *J* 7.3 Hz, CH_2O), 6.81 (1 H, d, *J* 7.6 Hz, Ar), 6.89 (1 H, d, *J* 7.6 Hz, Ar), 7.72 (2 H, td, *J* 5.8, 2.7 Hz, Ar), 7.84-7.88 (2 H, m, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ=12.5, 16.3, 19.8, 29.4, 35.6, 69.9, 123.1, 125.1, 127.8, 127.9, 129.4, 132.1, 133.8, 135.7, 155.7, 168.2; HRMS-ESI *m/z* calcd. for $[C_{20}H_{21}NO_3+H]^+$: 324.1594, found: 324.1588.

ii) 3-(2,3,6-Trimethylphenoxy)propan-1-amine (35). Prepared from **34** and purification, as described for **33**, afforded the title compound (513 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.95 (2 H, quintet, *J* 6.6 Hz, C**H**$_2$$CH_2O$), 2.18 (3 H, s, CH_3), 2.23 (3 H, s, CH_3), 2.25 (3 H, s, CH_3), 2.99 (2 H, t, *J* 6.9 Hz, CH_2N), 3.80 (2 H, t, *J* 6.2 Hz, CH_2O), 6.82 (1 H, d, *J* 7.6 Hz, Ar), 6.90 (1 H, d, *J* 7.6 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ=12.5, 16.3, 20.0, 34.3, 39.7, 70.5, 125.2, 127.9, 128.1, 129.5, 135.8, 155.8; HRMS-ESI *m/z* calcd. for $[C_{12}H_{19}NO+H]^+$: 194.1539, found: 194.1541.

iii) 2,6-Di-*tert*-butyl-4-((3-(2,3,6-trimethylphenoxy)propylamino)methyl)phenol (12). Method A1 applied to **35** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde followed by purification Protocol A (MeOH/H_2O) afforded the title compound (244 mg, 58%). ^{1}H NMR (400 MHz, CD_3OD): δ=1.44 (18 H, s, 2 × $C(CH_3)_3$), 2.14 (3 H, s, CH_3), 2.18-2.26 (8 H, m, 2 × CH_3,C**H**$_2$$CH_2N$), 3.33-3.36 (2 H, m, CH_2N), 3.81 (2 H, t, *J* 5.8 Hz, CH_2O), 4.18 (2 H, s, $ArCH_2N$), 6.80 (1 H, d, *J* 7.7 Hz, Ar), 6.87 (1 H, d, *J* 7.7 Hz, Ar), 7.34 (2 H, s, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=12.6, 16.5, 19.9, 28.0, 30.6, 35.6, 46.1, 52.9, 70.3, 123.0, 126.6, 128.0, 128.8, 129.0, 130.1, 137.0, 140.0, 156.3, 156.6; HRMS-ESI *m/z* calcd. for $[C_{27}H_{41}NO_2+H]^+$: 412.3210, found: 412.3211.

Preparation of 2,6-di-*tert*-butyl-4-((3-(2,4,6-trimethylpyrimidin-5-yloxy)propylamino) methyl) phenol (13).

i) *N*-(3-(2,4,6-Trimethylpyrimidin-5-yloxy)propyl)phthalimide (36). Prepared from 2,4,6-trimethylpyrimidinol and *N*-(3-bromopropyl)phthalimide as described for **34**, and purification by flash chromatography (7:3:0.1 EtOAc/petroleum spirits/Et_3N) gave the title compound (301.9 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.16 (2 H, quintet, *J* 6.8 Hz, C**H**$_2$$CH_2O$), 2.39 (6 H, s, 2 × CH_3), 2.55 (3 H, s, CH_3), 3.82 (2 H, t, *J* 6.2 Hz, CH_2N), 3.90 (2 H, t, *J* 7.1 Hz, CH_2O), 7.66-7.70 (2 H, m, Ar), 7.78-7.83 (2 H, m, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ=19.0, 25.3, 29.5, 35.3, 70.9, 123.4, 132.1, 134.1, 148.4, 159.6, 162.0, 168.3; HRMS-ESI *m/z* calcd. for $[C_{18}H_{19}N_3O_3+H]^+$: 326.1499, found: 326.1501.

ii) 3-(2,4,6-Trimethylpyrimidin-5-yloxy)propan-1-amine (37). Prepared from **36** and purification, as per **33**, afforded the title compound (82.7 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.04 (2 H, m, C**H**$_2$$CH_2O$), 2.41 (6 H, s, 2 × CH_3), 2.59 (3 H, s, CH_3), 3.06 (2 H, t, *J* 7.0 Hz, CH_2N), 3.85 (2 H, t, *J* 6.0 Hz, CH_2O); HRMS-ESI *m/z* calcd. for $[C_{10}H_{17}N_3O+H]^+$: 196.1444, found: 196.1442.

iii) 2,6-Di-*tert*-butyl-4-((3-(2,4,6-trimethylpyrimidin-5-yloxy)propylamino)methyl) phenol (13). Method A1 applied to **37** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde followed by purification Protocol B (MeOH/Et_2O) afforded the title compound (65.2 mg, 59%). ^{1}H NMR (400 MHz, CD_3OD): δ=1.45 (18 H, s, 2 × $C(CH_3)_3$), 2.34-2.37 (2 H, m, C**H**$_2$$CH_2N$), 2.68 (6 H, s, 2 × $ArCH_3$), 2.79 (3 H, s, $ArCH_3$), 3.31-3.36 (2 H, obscured m, CH_2N), 4.15 (2 H, t, *J* 6.0 Hz, CH_2O), 4.18 (2 H, s, $ArCH_2N$), 7.35 (2 H, s, Ar); HRMS-ESI *m/z* calcd. for $[C_{25}H_{39}N_3O_2+H]^+$: 414.3115, found: 414.3115.

Preparation of 2,6-di-*tert*-butyl-4-((3-(2-isopropyl-4,6-dimethylpyrimidin-5-yloxy) propylamino) methyl)phenol (14).

i) *N*-(3-(2-Isopropyl-4,6-dimethylpyrimidin-5-yloxy)propyl)phthalimide (38) Prepared from 2-isopropyl-4,6-dimethylpyrimidinol and *N*-(3-bromopropyl)phthalimide, as per **32**, and purification by flash chromatography (20-30% EtOAc/petroleum spirits) gave the title compound (422 mg 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.28 (6 H, d, *J* 7.0 Hz, $CH(CH_3)_2$), 2.21 (2 H, quintet, *J* 6.8 Hz, C**H**$_2$$CH_2N$), 2.45 (6 H, s, 2 × $ArCH_3$), 3.11 (1 H, septet, *J* 7.0 Hz, C**H**$(CH_3)_2$), 3.87, 3.95 (4 H, 2 × t, *J* 6.2 Hz, CH_2N,CH_2O), 7.71-7.76, 7.84-7.89 (4 H, 2 × m, Ar);^{13}C NMR (100 MHz, $CDCl_3$): δ=19.5, 22.4, 29.9, 35.8, 37.5, 71.2, 123.8, 132.6, 134.5, 148.8, 159.9, 168.8, 169.9; HRMS-ESI *m/z* calcd. for $[C_{20}H_{23}N_3O_3+H]^+$: 354.1818, found: 354.1813.

ii) 3-(2-Isopropyl-4,6-dimethylpyrimidin-5-yloxy)propan-1-amine (39). Prepared from **38** and purification, as per **33**, gave the title compound (245 mg, 94%). ^{1}H NMR (400 MHz, CD_3OD): δ=1.26 (6 H, d, *J* 7.0 Hz, $CH(CH_3)_2$), 1.96 (2 H, quintet, *J* 6.8 Hz, C**H**$_2$$CH_2N$), 2.44 (6 H, s, 2 × $ArCH_3$), 3.06 (1 H, septet, *J* 7.0 Hz, C**H**$(CH_3)_2$), 3.28 (2 H, t, *J* 6.2 Hz, CH_2N), 3.90 (4 H, t, *J* 6.2 Hz, CH_2O);^{13}C NMR (100 MHz, CD_3OD): δ=18.8, 22.2, 34.2, 37.9, 39.7, 72.6, 149.8, 161.4, 170.3; HRMS-ESI *m/z* calcd. for $[C_{12}H_{21}N_3O+H]^+$: 224.1757, found: 244.1763.

iii) 2,6-Di-*tert*-butyl-4-((3-(2-isopropyl-4,6-dimethylpyrimidin-5-yloxy)propylamino) methyl)phenol (14). Method A1 applied to **39** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde, followed by purification Protocol B (MeOH/H_2O) afforded the title compound (306 mg, 57%). ^{1}H NMR (500 MHz, CD_3OD): δ=1.40 (6 H, d, *J* 6.8 Hz, $CH(CH_3)_2$), 1.45 (18 H, s, 2 × $C(CH_3)_3$), 2.27-2.32 (2 H, m, CH_2CH_2N), 2.68 (6 H, s, 2 × $ArCH_3$), 3.22-3.28 (3 H, obscured multiplet, $CH(CH_3)_2$,CH_2N), 4.14 (2 H, t, *J* 6.0 Hz, CH_2O), 4.18 (2 H, s, $ArCH_2N$), 7.34 (2 H, s, Ar); ^{13}C NMR (125 MHz, CD_3OD): δ=18.3, 21.3, 27.9, 30.7, 35.3, 35.7, 45.4, 53.1, 72.6, 123.1, 128.0, 140.0, 150.3, 156.6, 165.5; HRMS-ESI *m/z* calcd. for $[C_{27}H_{43}N_3O_2+H]^+$: 442.3433, found: 442.3433.

4-((3-(2,6-Dimethylphenoxy)propylamino)methyl)phenol (15). Method A1 applied to 3-(2,6-dimethylphenoxy)propylamine[28] and 4-hydroxybenzaldehyde, followed by purification Protocol B (MeOH/H_2O) afforded the title compound (156 mg, 42%). ^{1}H NMR (500 MHz, CD_3OD): δ=2.19-2.25 (8 H, m, 2 × CH_3,C**H**$_2$$CH_2N$), 3.33-3.36 (2 H, obscured m, CH_2N), 3.88 (2 H, t, *J* 5.9 Hz, CH_2O), 4.19 (2 H, s, $ArCH_2N$), 6.87-6.93 (3 H, m, Ar), 7.01 (2 H, d, *J* 7.4 Hz, Ar), 7.36-7.38 (2 H, m, Ar); ^{13}C NMR (125 MHz, CD_3OD): δ=16.4, 28.1, 46.1, 52.1, 70.0, 116.9, 122.9, 125.3, 130.0, 131.7, 132.6, 156.6, 160.0; HRMS-ESI *m/z* calcd. for $[C_{18}H_{23}NO_2+H]^+$: 286.1807, found: 286.1804.

2,6-Dichloro-4-((3-(2,6-dimethylphenoxy)propylamino)methyl)phenol (16). Method A1 applied to 3-(2,6-dimethylphenoxy)propylamine[28] and 3,5-dichloro-4-hydroxybenzaldehyde, followed by purification using Protocol B (MeOH/H_2O) afforded the title compound (318 mg, 64%). ^{1}H

NMR (400 MHz, CD_3OD): δ=2.19-2.24 (8 H, m, 2 × CH_3,C**H**$_2$$CH_2$N), 3.33-3.35 (2 H, m, CH_2N), 3.87 (2 H, t, *J* 5.9 Hz, CH_2O), 4.19 (2 H, s, ArCH_2N), 6.89 (1 H, t, *J* 7.5 Hz, Ar), 6.98-7.00 (2 H, m, Ar), 7.53 (2 H, s, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=16.4, 28.1, 46.4, 50.9, 69.9, 123.82, 123.84, 124.8, 125.2, 130.0, 131.5, 131.7, 152.0, 156.6; HRMS-ESI *m/z* calcd. for $[C_{18}H_{21}Cl_2NO_2+H]^+$: 354.1022, found: 354.1027.

***N*-Benzyl-3-(2,6-dimethylphenoxy)propan-1-amine (17).** Method A1 applied to 3-(2,6-dimethylphenoxy)propylamine[28] and benzaldehyde, followed by purification using Protocol B (MeOH/H_2O) afforded the title compound (135 mg, 37%). ^{1}H NMR (500 MHz, CD_3OD): δ=2.16-2.22 (2 H, m, C**H**$_2$$CH_2$N), 2.23 (6 H, 2 × CH_3), 3.34 (2 H, t, *J* 6.4 Hz, CH_2N), 3.87 (2 H, t, *J* 5.8 Hz, CH_2O), 4.26 (2 H, s, ArCH_2N), 6.90 (1 H, t, *J* 6.0 Hz, Ar), 7.00 (2 H, m, Ar), 7.45-7.53 (5 H, m, Ar); ^{13}C NMR (125 MHz, CD_3OD): δ=16.4, 28.1, 46.5, 52.4, 70.0, 125.3, 130.0, 130.3, 130.7, 131.1, 131.7, 132.5, 156.6; HRMS-ESI *m/z* calcd. for $[C_{18}H_{23}NO+H]^+$: 270.1857, found: 270.1857.

***N*-(4-((3-(2,6-Dimethylphenoxy)propylamino)methyl)phenyl)acetamide (18).** Method B applied to 3-(2,6-dimethylphenoxy)propylamine[28] and 4-acetamidobenzaldehyde, followed by purification Protocol B (MeOH/Et_2O) afforded the title compound (82.2 mg, 45%). ^{1}H NMR (500 MHz, CD_3OD): δ=2.14 (3 H, NHAc), 2.18-2.24 (8 H, m, 2 × CH_3,C**H**$_2$$CH_2$N), 3.35 (2 H, t, *J* 7.9 Hz, CH_2N), 3.87 (2 H, t, *J* 5.8 Hz, CH_2O), 4.24 (2 H, s, ArCH_2N), 6.90 (1 H, t, *J* 7.5 Hz, Ar), 7.00 (2 H, d, *J* 7.5 Hz, Ar), 7.47 (2 H, d, *J* 8.5 Hz, Ar), 7.68 (2 H, d, *J* 8.5, Hz Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=16.42, 16.43, 23.89, 23.91, 28.1, 46.3, 52.0, 70.0, 121.4, 125.3, 127.6, 130.0, 131.69, 131.73, 141.4, 156.6, 171.8; HRMS-ESI *m/z* calcd. for $[C_{20}H_{26}N_2O_2+H]^+$: 327.2067, found: 327.2068.

Preparation of 2,6-di-*tert*-butyl-4-((3-(2,6-dimethyl-4-(thiophen-2-yl)phenoxy) propylamino)methyl)phenol (19).

i) 2,6-Dimethyl-4-(thiophen-2-yl)phenol (40). Pd(dppt)Cl_2 was added to a degassed (N_2) mixture of 3,5-dimethyl-4-hydroxyphenyl boronic acid pinacol ester (1.00 g, 4.0 mmol), 2-bromothiophene (853 mg, 5.20 mmol), K_3PO_4 (1.8 g, 8.48 mmol) and dry DMF (30 mL). The mixture was heated at 100 °C with stirring for 16 h, filtered through a silica plug rinsed with EtOAc, and the filtrate was concentrated under reduced pressure. The residue was purified by flash chromatography (10% EtOAc/petroleum spirits) to give the title compound. ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.25 (6 H, s, 2 × CH_3), 7.00 (1 H, m, Ar), 7.13-7.18 (2 H, m, Ar), 7.21 (2 H, s, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ=16.1, 122.00, 123.6, 123.7, 126.6, 126.9, 126.9, 128.0, 144.8, 152.1; HRMS-ESI *m/z* calcd. for $[C_{12}H_{12}OS+H]^+$: 205.0687, found: 205.0684.

ii) *N*-(3-(2,6-Dimethyl-4-(thiophen-2-yl)phenoxy)propyl)phthalimide (41). Prepared from **40**, as per **32**, and purification by flash chromatography (15-20% EtOAc/petroleum spirits) gave the title compound (320 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.12 (2 H, quintet, *J* 6.8 Hz, C**H**$_2$$CH_2$N), 2.30 (6 H, s, 2 × CH_3), 3.87, 3.97 (4 H, 2 × t, *J* 6.2 Hz, CH_2N,CH_2O), 7.02-7.06 (1 H, m, Ar), 7.18-7.25 (4 H, m, Ar), 7.70-7.74, 7.84-7.88 (4 H, 2 × m, Ar);^{13}C NMR (100 MHz, CD_3OD): δ=16.6, 29.6, 35.7, 70.0, 122.6, 123.4, 124.3, 126.6, 128.0, 130.1, 131.3, 132.3, 134.1, 144.4, 155.8, 168.5.

iii) 3-(2,6-Dimethyl-4-(thiophen-2-yl)phenoxy)propan-1-amine (42). Prepared from **41** and purification, as per **33**, gave the title compound (123 mg, 91%). ^{1}H NMR (400 MHz, CD_3OD): δ=1.99 (2 H, quintet, *J* 6.8 Hz, C**H**$_2$$CH_2$N), 2.30 (6 H, s, 2 × CH_3), 2.95 (2 H, t, *J* 6.2 Hz, CH_2N), 3.87 (2 H, t, *J* 6.2 Hz, CH_2O), 7.04 (1 H, m, Ar), 7.25-7.34 (4 H, m, Ar);^{13}C NMR (100 MHz, CD_3OD): δ=16.4, 33.1, 39.3, 70.3, 122.6, 124.3, 126.6, 128.0, 130.1, 131.4, 144.3, 155.5; HRMS-ESI *m/z* calcd. for $[C_{15}H_{19}NOS+H]^+$: 262.1266, found: 262.1263.

iv) 2,6-Di-*tert*-butyl-4-((3-(2,6-dimethyl-4-(thiophen-2-yl)phenoxy)propylamino)methyl) phenol (19). Method A1 applied to **42** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde, followed by purification Protocol B (MeCN/H_2O) afforded the title compound (68 mg, 21%). ^{1}H NMR (500 MHz, CD_3OD): δ=1.45 (18 H, s, 2 × C(CH_3)$_3$), 2.19-2.25 (2 H, m, C**H**$_2$$CH_2$N), 2.27 (6 H, s, 2 × CH_3), 3.32-3.36 (2 H, m, CH_2N), 3.90 (2 H, t, *J* 5.8 Hz, CH_2O), 4.18 (2 H, s, ArCH_2N), 7.05 (1 H, dd, *J* 5.1, 3.6 Hz, Ar), 7.26-7.32 (6 H, m, Ar); ^{13}C NMR (125 MHz, CD_3OD): δ=16.5, 28.1, 30.6, 35.6, 46.1, 53.0, 70.2, 123.0, 123.8, 125.4, 127.4, 127.9, 129.0, 131.9, 132.4, 140.1, 145.1, 156.2, 156.7; HRMS-ESI *m/z* calcd. for $[C_{30}H_{41}NO_2S+H]^+$: 490.2936, found: 480.2929.

Preparation of 2,6-di-*tert*-butyl-4-((3-(2,6-dimethyl-4-(thiophen-3-yl))propylamino) methyl)phenol (20).

i) 2,6-Dimethyl-4-(thiophen-3-yl)phenol (43). Pd(dppt)Cl_2 was added to a degassed (N_2) mixture of 3,5-dimethyl-4-hydroxyphenyl boronic acid pinacol ester (1.00 g, 4.0 mmol), 2-bromothiophene (853 mg, 5.20 mmol), K_3PO_4 (1.80 g, 8.48 mmol) and dry DMF (30 mL). The mixture was heated at 110 °C with stirring for 16 h, cooled to r.t and then concentrated under reduced pressure. The residue was partitioned between EtOAc and 1M aq. HCl, the organic layer was separated, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude material was crystallized from petroleum spirits to give the title compound (373 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.19 (6 H, s, 2 × CH_3), 7.20-7.26 (5 H, m, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ=16.1, 118.8, 123.4, 126.0, 126.4, 126.9, 128.4, 142.4, 151.7; HRMS-ESI *m/z* calcd. for $[C_{12}H_{12}OS+H]^+$: 205.0687, found: 205.0684.

ii) *N*-(3-(2,6-Dimethyl-4-(thiophen-3-yl)phenoxy)propyl)phthalimide (44). Prepared from **43**, as per **32**, and purification by flash chromatography (15% EtOAc/petroleum spirits) afforded the title compound (309 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.22 (2 H, quintet, *J* 6.8 Hz, C**H**$_2$$CH_2$N), 2.31 (6 H, s, 2 × CH_3), 3.88, 3.95 (4 H, 2 × t, *J* 6.2 Hz, CH_2N,CH_2O), 7.02-7.06 (1 H, m, Ar), 7.18-7.25 (4 H, m, Ar), 7.70-7.74, 7.84-7.88 (4 H, 2 × m, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=16.6, 29.6, 35.7, 70.0, 119.6, 123.2, 126.0, 126.5, 127.0, 131.2, 131.5, 132.2, 134.0, 142.2, 155.4, 168.5.

iii) 3-(2,6-Dimethyl-4-(thiophen-3-yl)phenoxy)propan-1-amine (45). Prepared from **44** and purification, as per **32**, afforded the title compound (123 mg, 91%). ^{1}H NMR (400 MHz, CD_3OD): δ=1.98 (2 H, quintet, *J* 6.8 Hz, C**H**$_2$$CH_2$N), 2.29 (6 H, s, 2 × CH_3), 2.94 (2 H, t, *J* 6.2 Hz, CH_2N), 3.85 (2 H, t, *J* 6.2 Hz, CH_2O), 7.34 (2 H, s, Ar), 7.36-7.48 (3 H, m, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=16.5, 33.8, 40.0, 71.4, 120.4, 127.0, 127.3, 127.8, 132.1, 132.9, 143.4, 157.3; HRMS-ESI *m/z* calcd. for $[C_{15}H_{19}NOS+H]^+$: 262.1266, found: 262.1261.

iv) 2,6-Di-*tert*-butyl-4-((3-(2,6-dimethyl-4-(thiophen-3-yl)phenoxy)propylamino)methyl) phenol (20). Method A1 applied to **45** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde, followed by purification Protocol B (MeOH/H_2O) afforded the title compound (105 mg, 49%). ^{1}H NMR (500 MHz, CD_3OD): δ=1.45 (18 H, s, 2 × C(CH_3)$_3$), 2.19-2.25 (2 H, m, C**H**$_2$$CH_2$N), 2.28 (6 H, s, 2 × CH_3), 3.35 (2 H, m, CH_2N), 3.90 (2 H, t, *J* 5.8 Hz, CH_2O), 4.18 (2 H, s, ArCH_2N), 7.32 (4 H, d, *J* 8.7 Hz, Ar), 7.37-7.38 (1 H, m, Ar), 7.43 (1 H, dd, *J* 5.0, 3.0 Hz, Ar), 7.49 (1 H, dd, *J* 2.9, 1.3, Ar); ^{13}C NMR (400 MHz, CD_3OD): δ=16.7, 28.0, 30.6, 35.6, 46.0, 52.9, 70.2, 120.5, 123.0, 127.0, 127.1, 127.8, 128.0, 132.0, 133.2, 139.9, 143.1, 155.8, 156.5; HRMS-ESI *m/z* calcd. for $[C_{30}H_{41}NO_2S+H]^+$: 480.2936, found: 480.2945.

Preparation of 2,6-Di-*tert*-butyl-4-((3-(3,5-dimethyl-1*H*-pyrazol-4-yloxy)propylamino) methyl)phenol (21).

i) *N*-(3-(3,5-Dimethyl-1*H*-pyrazol-4-yloxy)propyl)phthalimide (46). Prepared from *N*-(3-bromopropyl)phthalimide and 3,5-dimethyl-1*H*-pyrazol-4-ol,[29] as per **32**, and purification by flash chromatography (90% EtOAc/petroleum spirits) afforded the title compound (290 mg, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.07 (2 H, dd, *J* 13.6, 6.4 Hz, CH_2CH_2N), 2.19 (6 H, s, 2 × CH_3), 3.86 (4 H, td, *J* 6.6, 4.7 Hz, CH_2N,CH_2O), 7.67 (2 H, dd,

J 5.4, 3.1, Ar), 7.81 (2 H, dd, *J* 5.5, 3.1 Hz, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ=9.8, 29.2, 35.4, 72.0, 123.2, 132.1, 140.0, 134.6, 138.9, 168.4; HRMS-ESI *m/z* calcd. for $[C_{16}H_{17}N_3O_3+H]^+$: 300.1343, found: 300.1341.

ii) 3-(3,5-Dimethyl-1*H*-pyrazol-4-yloxy)propan-1-amine (47). A mixture of the phthalimide **46** (192 mg, 0.644 mmol), hydrazine hydrate (193 mg, 3.86 mmol) and ethanol (11 mL) was heated in an oil bath at 100 °C for 16 h under N_2. After cooling to 0 °C the mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was triturated with 14:5:1 CH_2Cl_2/MeOH/H_2O, the liquid fraction was concentrated to give enriched (~90% pure by ^{1}H NMR) amine, which was used in subsequent reactions without further purification (94.8 mg, 87%). ^{1}H NMR (500 MHz, CD_3OD): δ=1.85 (2 H, quint, *J* 7.0 Hz, C**H**$_2$CH$_2$N), 2.15 (6 H, s, 2 × CH_3), 2.85 (2 H, t, *J* 7.0 Hz, CH_2N), 3.88 (2 H, t, CH_2O); ^{13}C NMR (125 MHz, CD_3OD): δ=9.6, 33.9, 39.8, 73.9, 135.6 (2 C, br s), 140.1; HRMS-ESI *m/z* calcd. for $[C_8H_{15}N_3O+H]^+$: 170.1288, found: 170.1301.

iii) 2,6-Di-*tert*-butyl-4-((3-(3,5-dimethyl-1*H*-pyrazol-4-yloxy)propylamino)methyl)phenol (21). Method A1 applied to **47** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde, followed by purification by Protocol A (MeOH/Et_2O) afforded the title compound (210 mg, 85%). ^{1}H NMR (400 MHz, CD_3OD): δ=1.45 (18 H, s, 2 × C(CH_3)$_3$), 2.16-2.22 (2 H, m, CH_2CH_2N), 2.35 (6 H, s, 2 × NCCH_3), 3.26-3.30 (2 H, m, CH_2N), 4.08 (2 H, t, *J* 6.0 Hz, CH_2O), 4.17 (2 H, s, ArCH_2N), 7.35 (2 H, s, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=8.8, 27.8, 30.6, 35.6, 45.6, 53.0, 73.2, 123.1, 128.0, 137.7, 140.0, 156.6; HRMS-ESI *m/z* calcd. for $[C_{23}H_{36}N_3O_2+H]^+$: 388.2959, found: 388.2965).

Preparation of 2,6-Di-*tert*-butyl-4-((3-(1,3,5-trimethyl-1*H*-pyrazol-4-yloxy)propylamino) methyl)phenol (22).

i) 4-(3-Bromopropoxy)-1,3,5-trimethyl-1*H*-pyrazole (48). A mixture of 1,3,5-trimethyl-1*H*-pyrazole (320 mg, 2.91 mmol), *N*-(3-bromopropyl)phthalimide (587 mg, 2.91 mmol), K_2CO_3 (2.01 g, 14.3 mmol) and DMF (30 mL) was heated at 60 °C for 4 h. The mixture was cooled to r.t., filtered, concentrated under reduced pressure and partitioned between EtOAc and sat. aq. $NaHCO_3$ (20 mL of each). The organic layer was separated and the aqueous layer extracted with EtOAc (2 × 10 mL). The combined organic extracts were sequentially washed with sat. aq. $NaHCO_3$ (20 mL), H_2O (20 mL), then sat. aq. NaCl (20 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (4:4:1→3:5:1 toluene/CH_2Cl_2/acetone) to give the title compound (226 mg, 31%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=2.08 (3 H, s, CH_3), 2.10 (3 H, s, CH_3), 2.13 (2 H, C**H**$_2$CH$_2$N), 3.55 (2 H, t, *J* 6.0 Hz, CH_2Br), 3.57 (3 H, s, NCH_3), 3.83 (2 H, t, *J* 6.0 Hz, CH_2O); ^{13}C NMR (125 MHz, $CDCl_3$): δ=8.4, 10.7, 30.0, 32.9, 36.2, 128.9, 138.2, 138.7; HRMS-ESI *m/z* calcd. for $[C_9H_{15}BrN_2O+H]^+$: 247.0441, found: 247.0441.

ii) 4-(3-Azidopropoxy)-1,3,5-trimethyl-1*H*-pyrazole (49). Prepared from **48**, as per **28**, except that the reaction was heated at 60 °C for 5 h, then 100 °C for 2 h. Flash chromatography (4:1 toluene/acetone) gave the title compound (168 mg, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.83-1.89 (2 H, m, C**H**$_2$CH$_2$O), 2.06 (3 H, s, CH_3), 2.08 (3 H, s, CH_3), 3.44 (2 H, t, *J* 6.7 Hz, CH_2N), 3.55 (3 H, s, CH_3), 3.76 (2 H, t, *J* 6.0 Hz, CH_2O); ^{13}C NMR (100 MHz, $CDCl_3$): δ=8.3, 10.6, 29.3, 36.1, 48.1, 71.1, 128.7, 138.1, 138.8; HRMS-ESI *m/z* calcd. for $[C_9H_{17}N_3O+H]^+$: 210.1349, found: 210.1344).

iii) 3-(1,3,5-Trimethyl-1*H*-pyrazol-4-yloxy)propan-1-amine (50). Prepared from **49**, as per **29**, and purification by flash chromatography (93:5:2 toluene/MeOH/Et_3N) gave the title compound (110 mg, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.74 (2 H, quintet, *J* 6.6 Hz, C**H**$_2$CH$_2$O), 2.05 (3 H, s, CH_3), 2.07 (3 H, s, CH_3), 2.81 (2 H, t, *J* 6.9 Hz, CH_2N), 3.54 (3 H, s, CH_3), 3.76 (2 H, t, *J* 6.2 Hz, CH_2O); ^{13}C NMR (100 MHz, $CDCl_3$): δ=8.4, 10.7, 33.5, 36.18, 36.20, 39.1, 72.8, 128.8, 138.2, 139.1; HRMS-ESI *m/z* calcd. for $[C_9H_{19}NO+H]^+$: 184.1444, found: 184.1454.

iv) 2,6-Di-*tert*-butyl-4-((3-(1,3,5-trimethyl-1*H*-pyrazol-4-yloxy)propylamino)methyl) phenol (22). Method A1 applied to **50** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde, followed by purification by Protocol A (MeOH/H_2O) afforded the title compound (98.2 mg, 34%). ^{1}H NMR (400 MHz, CD_3OD): δ=1.44 (18 H, s, 2 × C(CH_3)$_3$), 2.16-2.18 (2 H, m, C**H**$_2$CH$_2$N), 2.29 (3 H, s, NCCH_3), 2.31 (3 H, s, NCCH_3), 3.25-3.29 (2 H, m, CH_2N), 3.83 (3 H, s, NCH_3), 4.03 (2 H, t, *J* 5.3 Hz, CH_2O), 4.16 (2 H, s, ArCH_2N), 7.33 (2 H, s, Ar); ^{13}C NMR (100 MHz, CD_3OD): δ=8.5, 9.1, 27.8, 30.6, 35.6, 36.1, 45.7, 53.1, 73.6, 123.1, 128.0, 137.4, 137.9, 140.0, 140.4, 156.6; HRMS-ESI *m/z* calcd. for $[C_{24}H_{38}N_3O_2+H]^+$: 402.3115, found: 402.3115.

Preparation of 2,6-Di-*tert*-butyl-4-((3-(2,4,6-trichlorophenoxy)propylamino)methyl) phenol (23).

i) *N*-(3-(2,4,6-Trichlorophenoxy)propyl)phthalimide (51). Prepared from *N*-(3-bromopropyl)phthalimide and 2,4,6-trichlorophenol, as per **32**, and purification by crystallization from EtOH afforded the title compound (1.84 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.19-2.26 (2 H, m, C**H**$_2$CH$_2$N), 3.96, 4.07 (4 H, 2 × t, *J* 6.5 Hz, CH_2N,CH_2O), 7.26 (2 H, s, Ar), 7.68-7.72 (2 H, m, Ar), 7.83 (2 H, m, Ar); ^{13}C NMR (100 MHz, $CDCl_3$): δ=29.2, 35.5, 71.5, 123.4, 128.8, 129.6, 130.1, 132.2, 134.0, 150.5, 168.4; HRMS-ESI *m/z* calcd. for $[C_{17}H_{12}Cl_3NO_3+H]^+$: 385.9932, found: 385.9925.

ii) 3-(2,4,6-Trichlorophenoxy)propan-1-amine (52).

Prepared from **51**, as per **31**, and purification by flash chromatography (17:2:1:0.4 EtOAc/MeOH/H_2O/Et_3N) gave the title compound (1.03 g, 91%). ^{1}H NMR (500 MHz, $CDCl_3$): δ=1.96-2.01 (2 H, m, C**H**$_2$CH$_2$N), 2.92 (2 H, t, *J* 7.0 Hz, CH_2N), 4.09 (2 H, t, *J* 6.0 Hz, CH_2O), 7.45 (2 H, d, *J* 0.6 Hz, Ar); ^{13}C NMR (125 MHz, $CDCl_3$): δ=34.0, 39.8, 73.1, 130.0, 130.8, 131.2, 151.9; HRMS-ESI *m/z* calcd. for $[C_9H_{10}Cl_3NO+H]^+$: 255.9877, found: 255.9880.

iii) 2,6-Di-*tert*-butyl-4-((3-(2,4,6-trichlorophenoxy)propylamino)methyl)phenol (23). Method A1 applied to **51** and 3,5-di-*tert*-butyl-4-hydroxybenzylaldehyde, followed by purification Protocol B (MeOH/Et_2O) afforded the title compound (580 mg, 97%). ^{1}H NMR (500 MHz, CD_3OD): δ=1.44 (18 H, s, 2 × C(CH_3)$_3$), 2.23-2.28 (2 H, m, C**H**$_2$CH$_2$N), 3.34-3.37 (2 H, m, C**H**$_2$N), 4.12 (2 H, t, *J* 5.7 Hz, CH_2O), 4.17 (2 H, s, ArCH_2N), 7.32 (2 H, s, Ar), 7.48 (2 H, s, Ar); ^{13}C NMR (125 MHz, CD_3OD): δ=27.8, 30.6, 35.6, 45.8, 53.0, 71.8, 122.9, 128.0, 130.1, 131.1, 131.3, 140.0, 151.2, 156.6; HRMS-ESI *m/z* calcd. for $[C_{24}H_{32}Cl_3NO_2+H]^+$: 472.1576, found: 472.1573).

***N*-(3,5-Di-*tert*-butylbenzyl)-3-(2,6-dimethylphenoxy)propan-1-amine (24).** A mixture of 3-(2,6-dimethylphenoxy)propan-1-amine (316 mg, 1.76 mmol), 3,5-di-*tert*-butylbenzyl bromide (250 mg, 0.88 mmol) and dry THF (25 mL) was heated at reflux for 24 h. The mixture was concentrated under reduced pressure, 10% aq. NaOH (50 mL) was added and the mixture extracted with EtOAc (2 × 50 mL). The combined organic extracts were washed with sat. aq. NaCl, dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by Protocol B (MeOH/H_2O) afforded the title compound (146 mg, 20%). ^{1}H NMR (500 MHz, CD_3OD): δ=1.34 (18 H, s, 2 × C(CH_3)$_3$), 2.20-2.25 (8 H, m, 2 × CH_3,C**H**$_2$CH$_2$N), 3.35-3.39 (2 H, m, CH_2N), 3.88 (2 H, t, *J* 5.8 Hz, CH_2O), 4.27 (2 H, s, ArCH_2N), 6.90 (1 H, t, *J* 7.5 Hz, Ar), 7.00 (2 H, dd, *J* 7.4, 0.5 Hz, Ar), 7.39 (2 H, d, *J* 1.8 Hz, Ar), 7.55 (1 H, t, *J* 1.8 Hz, Ar); ^{13}C NMR (125 MHz, CD_3OD): δ=16.4, 28.1, 31.8, 35.8, 46.5, 53.0, 70.0, 124.8, 125.2, 125.3, 130.0, 131.7, 131.8, 153.3, 156.6; HRMS-ESI *m/z* calcd. for $[C_{26}H_{39}NO+H]^+$: 382.3109, found: 382.3107.

3-(2,6-Dimethylphenoxy)-*N*-(4-(trifluoromethoxy)benzyl)propan-1-amine (25). Method A1 applied to 3-(2,6-dimethylphenoxy)propylamine and 4-methoxylbenzaldehyde, followed by purification using Protocol A (MeOH/H_2O) afforded the title compound (157 mg, 72%). ^{1}H NMR (500 MHz, CD_3OD): δ=2.22-2.26 (8 H, m, 2 × CH_3,C**H**$_2$CH$_2$N), 3.37-3.40 (2 H, m, CH_2N), 3.88 (2 H, t, *J* 5.9 Hz, CH_2O), 4.33 (2 H, s, ArCH_2N), 6.90 (1 H, t, *J* 7.5 Hz, Ar), 7.00 (2 H, d, *J* 7.4 Hz, Ar), 7.39-7.41 (2 H, m, Ar), 7.66-7.68 (2 H, m, Ar); ^{13}C NMR (125 MHz, CD_3OD): δ=16.4, 28.2, 46.6, 51.5,

69.9, 120.8 (1C, q, *J* 256.1 Hz, CF_3), 122.7, 125.3, 130.0, 131.7, 131.7, 133.2, 151.3, 156.6; HRMS-ESI *m/z* calcd. for $[C_{19}H_{22}F_3NO_2+H]^+$: 354.1675, found: 354.1675.

4-((3-(2,6-Dimethylphenoxy)propylamino)methyl)benzenesulfonamide (26). Method A1 applied to 3-(2,6-dimethylphenoxy)propylamine and 4-aminosulfonylbenzaldehyde, followed by purification using Protocol B ($MeOH/H_2O$) afforded the title compound (277 mg, 62%). ^{1}H NMR (500 MHz, CD_3OD): δ=2.27-2.37 (8 H, m, 2 × CH_3,C**H**$_2$$CH_2$N), 3.40 (2 H, t, *J* 8.0 Hz, CH_2N), 3.89 (2 H, t, *J* 6.0 Hz, CH_2O), 4.38 (2 H, s, ArCH_2N), 6.90 (1 H, t, *J* 7.5 Hz, Ar), 7.00 (2 H, d, *J* 7.5 Hz), 7.74 (2 H, d, *J* 8.0 Hz, Ar), 8.00 (2 H, d, *J* 8.0 Hz, Ar); ^{13}C NMR (125 MHz, CD_3OD): δ=16.4, 28.2, 46.8, 51.7, 69.9, 125.3, 128.0, 130.0, 131.7, 136.6, 146.4, 156.6; HRMS-ESI *m/z* calcd. for $[C_{18}H_{24}N_2O_3S+H]^+$: 349.1585, found: 349.1588.

In vitro metabolic stability

The metabolic stability assay was performed by incubating each test compound in liver microsomes at 37°C and a protein concentration of 0.4 mg/mL. The metabolic reaction was initiated by the addition of an NADPH-regenerating system and quenched at various time points over a 60 min incubation period by the addition of acetonitrile containing diazepam as internal standard. Control samples (containing no NADPH) were included (and quenched at 2, 30 and 60 minutes) to monitor for potential degradation in the absence of cofactor. The human liver microsomes used in this experiment were supplied by XenoTech, lot#1410230. Microsomal incubations were performed at a substrate concentration of 1 μM.

Data analysis: Species scaling factors from Ring *et al*.[30] were used to convert the in vitro CL_{int} (μL/min/mg) to an in vivo CL_{int} (mL/min/kg). Hepatic blood clearance and the corresponding hepatic extraction ratio (E_H) were calculated using the well stirred model of hepatic extraction in each species, according to the "in vitro $T_{1/2}$" approach described in Obach[31]. The E_H was then used to classify compounds as low (<0.3), intermediate (0.3-0.7), high (0.7-0.95) or very high (>0.95) extraction compounds. Predicted in vivo clearance values have not been corrected for microsomal or plasma protein binding. Species scaling calculations are based on two assumptions: 1) NADPH-dependent oxidative metabolism predominates over other metabolic routes (i.e. direct conjugative metabolism, reduction, hydrolysis, etc.), and; 2) rates of metabolism and enzyme activities in vitro are truly reflective of those that exist in vivo. If significant non-NADPH-mediated degradation is observed in microsome control samples, then assumption (1) is invalid and predicted clearance parameters are therefore not reported.

Voltage-gated Na^+ channels

Sodium channel activity was studied under contract at ChanTest (Ohio, USA) using a whole-cell patch clamp technique in CHO cells that were stably transfected with human $Na_V1.2$ (SCN2A) and $Na_V1.6$ (SCN8A) cDNAs were incorporated into an expression plasmid along with antibiotic resistance genes. Cells were cultured in Ham's F-12 supplemented with 10% fetal bovine serum, 100 U/mL penicillin G sodium, 100 μg/mL streptomycin sulfate and appropriate selection antibiotics. Before testing, cells in culture dishes were washed twice with Hank's Balanced Salt Solution and treated with Accutase for approximately 20 min. Immediately before use the cells were washed in HEPES-buffered physiological saline to remove the Accutase and re-suspended in HEPES-buffered physiological saline.

Test article solutions were prepared in a ChanTest proprietary HEPES-buffered solution and applied to the extracellular well of the Population Patch Clamp™ (PPC) planar electrode. The intracellular solution for the PPC planar electrode comprised a ChanTest proprietary HEPES-buffered physiological saline solution.

All experiments were conducted at ambient temperature. Eight concentrations were applied to naïve cells (n = 4, where n = the number of replicate wells/concentration) via steel needles of a multi-channel pipettor. Vehicle control was applied for a 5 min exposure interval (n = 8). To verify the sensitivity of the assay to block, lamotrigine and lidocaine were applied for a 10 min exposure interval (n = 4). All test and control solutions contained 0.3% DMSO and 0.05% Pluronic F-127. Each test article formulation was sonicated (Model 2510/5510, Branson Ultrasonics, Danbury, CT), at ambient room temperature for at least 20 min to facilitate dissolution.

Data acquisition and analyses were performed using the IonWorks QuattroTM or Barracuda system software (Molecular Devices Corporation, Union City, CA) according to protocols 1 or 2. The decrease in current amplitude after test article application was used to calculate the percent block relative to control at the various test pulses as follows:

$$\text{Block}(\%) = (1 - I_{TP,TA} / I_{TP,control}) \times 100\% \quad (1)$$

Concentration-response data were fit to the following equation:

$$\text{Block}(\%) = \%VC + ((100 - \%VC) / [1 + (C / IC_{50})^N]) \quad (2)$$

where *C* is the concentration of test article, IC_{50} is the concentration of the test article producing half-maximal inhibition, *N* is the Hill coefficient, %VC is the percentage of the current run-down (the mean current inhibition at the vehicle control) and Block(%) is the percentage of ion channel current inhibited at each concentration of a test article. Nonlinear least squares fits were solved with the XL*fit* add-in for Excel (Microsoft, Redmond, WA).

6 Hz psychomotor seizure model of partial epilepsy

Tests were conducted by the Epilepsy Therapy Screening Program (ETSP) through the National Institute of Neurological Disorders and Stroke (NINDS). The test protocol was that reported in Barton *et al*.[24]

Acknowledgements

We are grateful to the National Institute of Neurological Disorders and Stroke (NINDS) Epilepsy Therapy Screening Program (ETSP) for *in vivo* mouse studies of the antiseizure properties of compound **2**. We thank FastForward (USA) for project funding, and invaluable advice from Dr John Lowe.

Keywords: membrane proteins • ion channels • neurological agents • anticonvulsant • propylamine

References:

[1] C. A. Ahern, J. Payandeh, F. Bosmans, B. Chanda, *J. Gen. Physiol.* **2016**, *147*, 1-24.
[2] J. R. Deuis, A. Mueller, M. R. Israel, I. Vetter, *Neuropharmacology* **2017**, *127*, 87-108.
[3] S. K. Bagal, M. L. Chapman, B. E. Marron, R. Prime, R. I. Storer, N. A. Swain, *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3690-3699.
[4] R. J. Docherty, C. E. Farmer, *Handb. Exp. Pharmacol.* **2009**, 519-561.
[5] B. T. Priest, *Curr. Opin. Drug Discov. Devel.* **2009**, *12*, 682-692.
[6] J. H. Caldwell, K. L. Schaller, R. S. Lasher, E. Peles, S. R. Levinson, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 5616-5620.
[7] S. A. Buffington, M. N. Rasband, *Eur. J. Neurosci.* **2011**, *34*, 1609-1619.

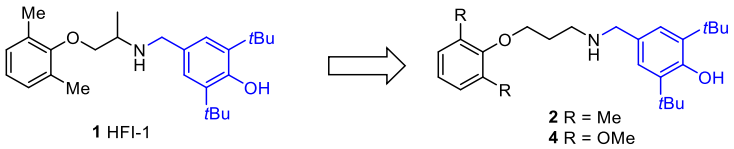
[8] A. Nardi, N. Damann, T. Hertrampf, A. Kless, *ChemMedChem* **2012**, *7*, 1712-1740.
[9] L. A. Clutterbuck, C. G. Posada, C. Visintin, D. R. Riddall, B. Lancaster, P. J. Gane, J. Garthwaite, D. L. Selwood, *J. Med. Chem.* **2009**, *52*, 2694-2707.
[10] P. E. Fenster, K. A. Comess, *Pharmacotherapy* **1986**, *6*, 1-9.
[11] C. Chabal, L. Jacobson, A. Mariano, E. Chaney, C. W. Britell, *Anesthesiology* **1992**, *76*, 513-517.
[12] J.-F. Desaphy, R. Carbonara, T. Costanza, D. Conte Camerino, *Exp. Neurol.* **2014**, *255*, 96-102.
[13] L. A. Foster, M. R. Johnson, J. T. MacDonald, P. I. Karachunski, T. R. Henry, D. R. Nascene, B. P. Moran, G. V. Raymond, *Pediatr. Neurol.* **2017**, *66*, 108-111.
[14] I. R. Carroll, K. M. Kaplan, S. C. Mackey, *J. Pain Symptom Manage.* **2008**, *35*, 321-326.
[15] J.-F. Desaphy, A. Dipalma, T. Costanza, R. Carbonara, M. M. Dinardo, A. Catalano, A. Carocci, G. Lentini, C. Franchini, D. C. Camerino, *Front. Pharmacol.* **2012**, *3*, 17-17.
[16] A. De Luca, F. Natuzzi, J. F. Desaphy, G. Loni, G. Lentini, C. Franchini, V. Tortorella, D. C. Camerino, *Mol. Pharmacol.* **2000**, *57*, 268-277.
[17] A. De Luca, S. Talon, M. De Bellis, J. F. Desaphy, C. Franchini, G. Lentini, A. Catalano, F. Corbo, V. Tortorella, D. Conte-Camerino, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2003**, *367*, 318-327.
[18] R. M. Weston, K. R. Subasinghe, V. Staikopoulos, B. Jarrott, *Neurochem. Res.* **2009**, *34*, 1816-1823.
[19] B. Jarrott, J. K. Callaway, W. R. Jackson, P. M. Beart, *Drug Dev. Res.* **1999**, *46*, 261-267.
[20] J. K. Callaway, A. J. Lawrence, B. Jarrott, *Neuropharmacology* **2003**, *44*, 787-800.
[21] J. K. Callaway, P. M. Beart, B. Jarrott, S. F. Giardina, *Br. J. Pharmacol.* **2001**, *132*, 1691-1698.
[22] G. E. Kirsch, *Drug Discov. Devel.* **2010**, 28-30.
[23] J. H. Kehne, B. D. Klein, S. Raeissi, S. Sharma, *Neurochem. Res.* **2017**, *42*, 1894-1903.
[24] M. E. Barton, B. D. Klein, H. H. Wolf, H. S. White, *Epilepsy Res.* **2001**, *47*, 217-227.
[25] W. Loscher, *Seizure* **2011**, *20*, 359-368.
[26] M. Mantegazza, G. Curia, G. Biagini, D. S. Ragsdale, M. Avoli, *Lancet Neurol.* **2010**, *9*, 413-424.
[27] B. Jarrott, **2009**.
[28] D. I. Barron, P. M. G. Bavin, G. J. Durant, I. L. Natoff, R. G. W. Spickett, D. K. Vallance, *J. Med. Chem.* **1963**, *6*, 705-711.
[29] P. J. Fagan, E. E. Neidert, M. J. Nye, M. J. O'Hare, W.-P. Tang, *Can. J. Chem.* **1979**, *57*, 904-912.
[30] B. J. Ring, J. Y. Chien, K. K. Adkison, H. M. Jones, M. Rowland, R. D. Jones, J. W. Yates, M. S. Ku, C. R. Gibson, H. He, R. Vuppugalla, P. Marathe, V. Fischer, S. Dutta, V. K. Sinha, T. Bjornsson, T. Lave, P. Poulin, *J. Pharm. Sci.* **2011**, *100*, 4090-4110.
[31] R. S. Obach, *Drug Metab. Dispos.* **1999**, *27*, 1350-1359.

10.1002/cmdc.201800781

WILEY-VCH

FULL PAPER

Entry for the Table of Contents



1 HFI-1

2 R = Me
4 R = OMe

New kids on the block: Achiral analogues of mexiletine were identified as selective blockers of human voltage gated sodium channel 1.2. Compound **2** was highly potent for $Na_V1.2$ and 500 times less potent in inhibiting $Na_V1.6$ channels; compound **4** was the most potent blocker but is non-selective against both channels. Compound **2** inhibited seizures induced by 6 Hz 44 mA electrical stimulation in a kindled mouse model of refractory epilepsy.