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Discovery of novel 4-phenyl-2-(pyrrolidinyl)nicotinamide derivatives as potent $Na_v1.1$ activators

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ABSTRACT

The voltage-gated sodium channel, $Na_v1.1$, is predominantly expressed in parvalbumin-positive fast spiking interneurons and has been genetically linked to Dravet syndrome. Starting from a high throughput screening hit isoxazole derivative **5**, modifications of **5** via combinations of IonWorks and Q-patch assays successfully identified the nicotinamide derivative **4**. Its increasing decay time constant (tau) of $Na_v1.1$ currents at 0.03 μM along with significant selectivity against $Na_v1.2$, $Na_v1.5$, and $Na_v1.6$ and acceptable brain exposure in mice was observed. Compound **4** is a promising $Na_v1.1$ activator that can be used to analyze pathophysiological functions of the $Na_v1.1$ channel towards treating various central nervous system diseases.

$Na_v1.1$ is a voltage-gated sodium channel (Na_v), comprising one pore-forming α-subunit encoded by *SCN1A* and two associated β-subunits encoded by *SCN1B-SCN4B*. $Na_v1.1$ as well as its subfamilies ($Na_v1.2$, $Na_v1.3$ and $Na_v1.6$), is predominantly expressed in the central nervous system (CNS).[1,2] $Na_v1.1$ is largely expressed in parvalbumin-positive fast spiking interneurons (FSINs) and is involved in membrane depolarization and action potential (AP) firing.[3] Therefore, loss of function of the $Na_v1.1$ channels could lead to disinhibition of excitatory pyramidal neurons causing various diseases of the CNS.[4–6] Dravet syndrome is a rare genetic epileptic encephalopathy, where more than 70% of patients have *de novo* heterozygous mutations of the *SCN1A* gene.[7] In these mutations, a loss of function of the $Na_v1.1$ channels has been reported.[8] The genetic link between Dravet syndrome patients and $Na_v1.1$ channels suggest that a brain penetrant $Na_v1.1$ activator could possess significant therapeutic potential for treating Dravet syndrome.[9,10] However, potent and selective $Na_v1.1$ activators have not been reported to date. Recently, a few $Na_v1.1$ activators have been reported by Lundbeck: a 2-methylbenzamide derivative (**1**),[11] AA43279 (**2**),[12] and Lu AE98134 (**3**)[13] (Fig. 1). The most recently developed activator, Lu AE98134 (**3**), increases the total area under the curve for the duration of the depolarizing pulse from 1 μM in $Na_v1.1$-expressing HEK cells, while issues of low selectivity against $Na_v1.5$ and moderate selectivity against $Na_v1.2$ were observed. Biologically, $Na_v1.5$ is a major cardiac sodium channel[14] and $Na_v1.2$ is dominantly expressed in excitatory neurons.[15,16] Therefore, high selectivity against $Na_v1.5$ and $Na_v1.2$ is preferable for drug candidates. On the other hand, the electrophysiology data regarding Lu AE98134 (**3**) reveals promising potency as a $Na_v1.1$ activator for increasing the excitability of FSINs. Herein, we report the discovery of a 4-phenyl-2-(pyrrolidinyl)nicotinamide derivative **4** as a highly potent $Na_v1.1$ activator with improved selectivity against $Na_v1.2$ and $Na_v1.5$ compared with previously reported $Na_v1.1$ activators.

To identify novel chemical lead compounds for $Na_v1.1$ activators, a high-throughput screening (HTS) was conducted at a single concentration (30 μM) using $Na_v1.1$-expressing CHO cells on the IonWorks platform followed by confirmation by Q-patch assays. From the HTS, isoxazole derivative **5** was identified as a hit compound which increased the decay time constant, tau value (179%), of $Na_v1.1$ currents relative to the control current at 10 μM without significant inhibition of the peak current (top peak current ratio = 86%, Table 1).[17] An initial structure–activity relationships (SAR) campaign identified pyrimidine derivative **6** with an increased tau value (198%) at 1 μM by Q-patch with lead-like physicochemical properties (LogD = 2.97 (pH7.4) and solubility > 100 μg/mL (pH6.8)). Parallel relationships between increasing tau values by the two methods were observed in **5** and **6**. Thus, we initially estimated the tau value of the tested compounds by Ion-

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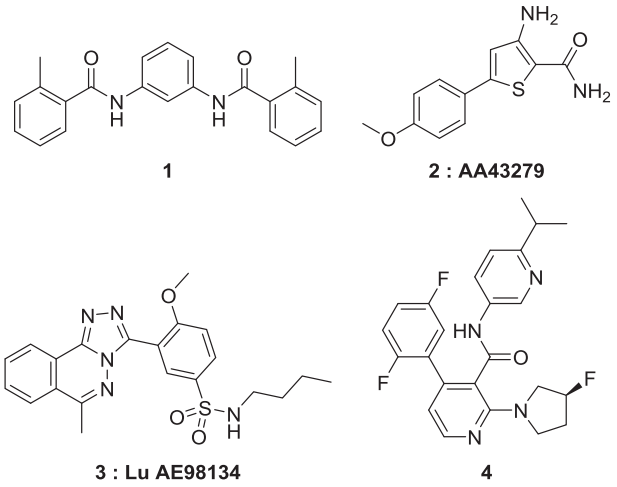


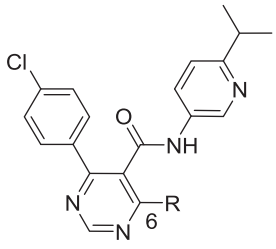
Fig. 1. Small Molecule Nav1.1 Activators.

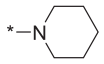
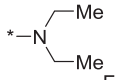
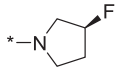
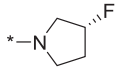
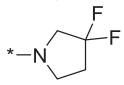
Works with moderate throughput (80–100 data points, e.g. 30 compounds with 3 concentrations per assay) followed by low throughput Q-patch tests (12 data points per assay) to evaluate precise tau values of the tested compounds.

Using **6** as a starting point, more potent compounds were investigated with increasing tau value at 10 nM. Initially, we investigated a cyclic amine substituent at the *C*-6 position of the pyrimidine ring of **6** (Table 2). Regarding the ring size at the *C*-6 position, the 5-membered pyrrolidine derivative **8** was preferable to a 6-, 7-, or 4-membered **9**. The ring-opened derivative **10** did not increase tau value compared with the corresponding cyclic **8**. Consequently, the introduction of fluorine atoms on the pyrrolidine ring of **8** successfully identified a (*S*)-3-fluoropyrrolidine substituent in **11** with a higher tau value (682% at 1 μM) by Q-patch than that of **6** (198% at 1 μM).

After identification of the (*S*)-3-fluoropyrrolidine moiety at the *C*-6 position of the pyrimidine ring, we replaced the 4-chlorophenyl moiety of **11** to further improve potency (Table 3). We initially removed the

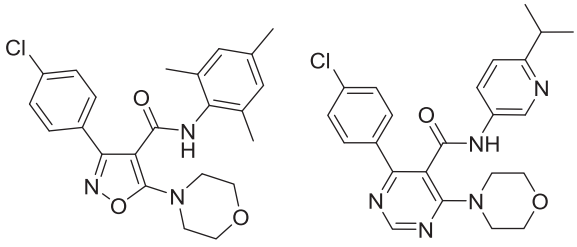
Table 2
SAR of the amine moiety at the *C*-6 position of the pyrimidine core.



Cmpd	R	hNa$_v$1.1 tau (%) by IonWorks[a]		hNa$_v$1.1 tau (%) by Q-patch[b]
		3 μM	10 μM	1 μM
7		110 (7)	105 (15)	ND[c]
8		140 (14)	257 (1 0 2)	ND
9		111 (9)	90 (18)	ND
10		96 (7)	74 (18)	ND
11		257 (51)[d]	342 (167)	682 (235)
12		145 (7)	162 (64)[d]	ND
13		184 (18)[d]	131 (31)	ND

[a] Tau values in IonWorks assays are averages of N = 5 unless noted. Standard deviation is provided in parentheses.
[b] Tau values in Q-patch experiments are averages of N = 4 unless noted. Standard deviation is provided in parentheses.
[c] ND: not determined.
[d] N = 4.

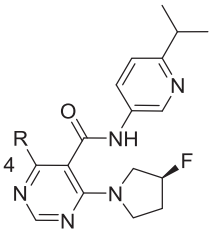
Table 1
Biological and Physicochemical Properties of HTS Hit Compound **5** and Lead Compound **6**.

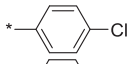
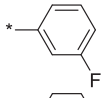
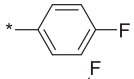
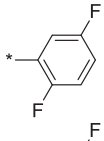
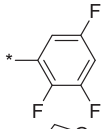
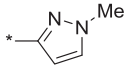


Cmpd	hNa$_v$1.1 tau (%) by IonWorks[a]			hNa$_v$1.1 tau (%) and top current ratio (%) by Q-patch[b]			LogD[e]	Solubility (μg/mL)[f]
	3 μM	10 μM	30 μM	1 μM	3 μM	10 μM		
5	99 (6)	102 (8)	138 (73)	113 (3) 92 (4)	120 (6)[c] 86 (6) [c]	179 (45)[d] 86 (9)[d]	3.43	0.90
6	258 (21)	370 (90)	555 (1 2 6)	198 (24) 113 (15)	731 (1 0 3) 115 (8)	774 (1 4 9) 131 (6)	2.97	> 100

[a] Tau values in IonWorks assays are averages of N = 5 unless noted. Standard deviation is provided in parentheses.
[b] Tau and top peak current values in Q-patch experiments are averages of N = 4 unless noted. Standard deviation is provided in parentheses.
[c] N = 5.
[d] N = 7.
[e] LogD at pH7.4.
[f] Solubility in pH6.8.

Table 3
SAR of aryl moiety at the *C*-4 position of the pyrimidine core.

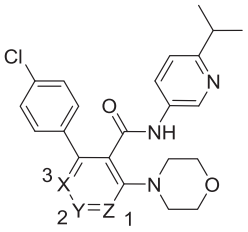


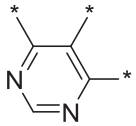
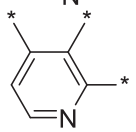
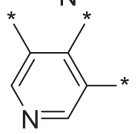
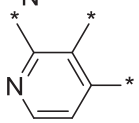
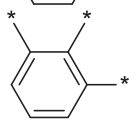
Cmpd	R	$hNa_v1.1$ tau (%) by IonWorks[a]		$hNa_v1.1$ tau (%) by Q-patch[b]	
		1 μM	3 μM	0.3 μM	1 μM
11		107 (10)	257 (51)[c]	170 (28)	682 (2 3 5)
14		102 (35)	302 (33)	255 (52)	332 (1 5 8)
15		135 (36)	391 (78)	711 (2 4 7)	1402 (2 1 3)
16		111 (7)	271 (44)	ND[d]	ND
17		93 (5)	197 (17)	ND	ND
18		260 (25)	714 (105)	853 (130)	1904 (74)
19		114 (14)	298 (35)	ND	ND
20		80 (3)	115 (10)	ND	ND
21		86 (5)	98 (8)	ND	ND
22		80 (4)[e]	87 (8)[e]	ND	ND

[a] Tau values in IonWorks assays are averages of N = 5 unless noted. Standard deviation is provided in parentheses.
[b] Tau values in Q-patch experiments are averages of N = 4 unless noted. Standard deviation is provided in parentheses.
[c] N = 4.
[d] ND: not determined.
[e] N = 8.

chlorine atom of **11** to yield **14**, which exhibited an increased tau value (225% at 0.3 μM) equivalent to **11** (170% at 0.3 μM) as determined by Q-patch assays. Next, fluorine atoms were introduced into the phenyl ring of **14** to provide **15–19**. Among them, the 2,5-difluoro substitution pattern significantly enhanced the tau value (853%) at 0.3 μM. On the other hand, replacement of the phenyl ring at the *C*-4 with furan rings (**20** and **21**) or a pyrazole ring (**22**) did not increase the tau value up to 3 μM in IonWorks assays. Thus, it was concluded that 2,5-difluorophenyl was the best substituent at the *C*-4 position.

Table 4
SAR of the core skeleton.

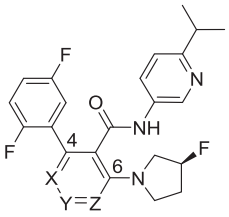


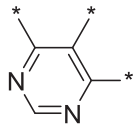
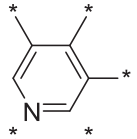
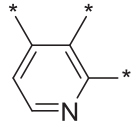
Cmpd	Core skeleton	$hNa_v1.1$ tau (%) by IonWorks[a]	
		3 μM	10 μM
6		258 (21)	370 (90)
23		374 (81)	767 (318)[b]
24		313 (46)	699 (370)
25		97 (5)	107 (24)
26		99 (10)	110 (29)

[a] Tau values in IonWorks assays are averages of N = 5 unless noted. Standard deviation is provided in parentheses.
[b] N = 4.

In parallel with optimization of the substituent at the *C*-4 and *C*-6 positions of the pyrimidine ring, the pyrimidine core of **6** was replaced with three types of pyridine rings (one of X or Y or Z = N) and a benzene ring (X, Y , Z = CH, Table 4). The pyridine derivatives **23** and **24** exhibited higher tau values than **6**, whereas the pyridine derivative **25** and benzene derivative **26** did not increase the tau value up to 10 μM. This could suggest that the presence of a hydrogen bonding acceptor such as a nitrogen atom on the 1- or 2-position of the 6-membered core is important for activity as a $Na_v1.1$ activator. By replacing the pyrimidine core of **6**, we identified the two promising pyridine ring candidates (Y = N or Z = N) as a core skeleton.

Table 5
SAR of the derivatives of compound **4**.



Cmpd	Core skeleton	hNa$_v$1.1 tau (%) by IonWorks[a]		hNa$_v$1.1 tau (%) by Q-patch[b]	
		0.3 μM	1 μM	0.03 μM	0.1 μM
18		135 (19)	260 (25)	124 (13)	193 (83)
27		92 (12)	124 (6)	ND[c]	ND
4		176 (32)	492 (31)	255 (62)	1204 (290)

[a] Tau values in IonWorks assays are averages of N = 5 unless noted. Standard deviation is provided in parentheses.
[b] Tau values in Q-patch experiments are averages of N = 4 unless noted. Standard deviation is provided in parentheses.
[c] ND: not determined.

Finally, the optimal 2,5-difluorophenyl substituent at the *C*-4 position and (*S*)-3-fluoropyrrolidine substituent at the *C*-6 position were incorporated into the promising pyridine core (Y = N or Z = N) to afford **27** and **4** (Table 5). While **27** did not increase the tau value at 0.3 μM as determined by IonWorks, **4** exhibited a higher tau value (255%) than **18** at 0.03 μM, as determined by Q-patch assay. The identification of **4** with 10 nM order activity encouraged us to further evaluate the *in vivo* brain pharmacokinetics (PK) and subtype selectivity against other sodium channels.

Table 6
PK Profiles in Mice and MDR1 Permeability of **4**.

Exposure in plasma and brain in mice[a]					MDR1 ER[d]
Plasma (ng/mL)	Brain (ng/g)	K_p[b]	$F_{u,b}$[c]	Free brain conc. (nM)	1 μM
968	193	0.20	0.029	13	1.0

[a] Exposure was measured at 1 h after 30 mg/kg intraperitoneal (i.p.) administration.
[b] Ratio of the total concentration in brain and that in plasma.
[c] Brain protein binding ratio.
[d] MDR1 efflux ratios (ER) in human MDR1-overexpressing LLC-PK1 cells at 1 μM of substrate.

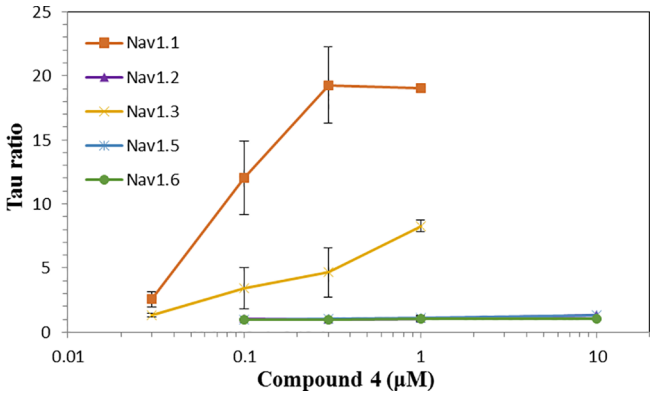


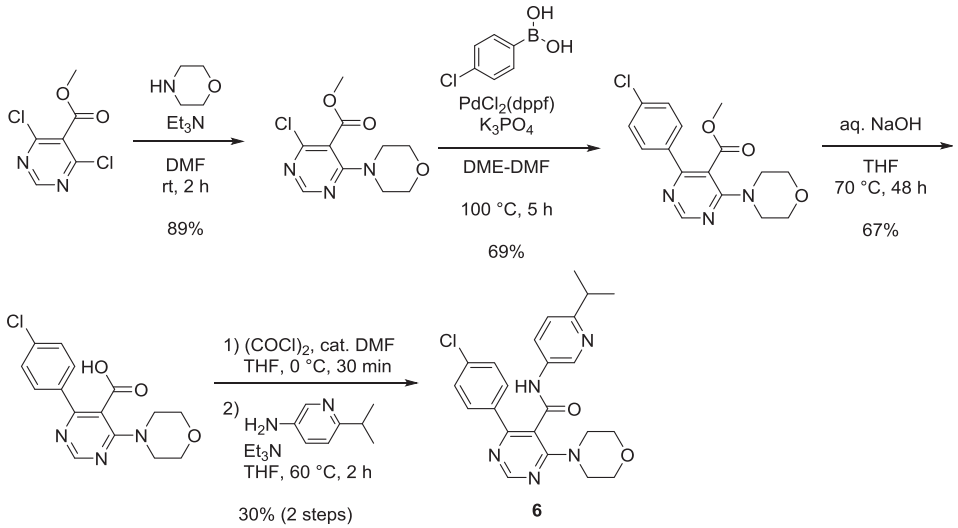
Fig. 2. Subtype selectivity of **4** towards other Na$_v$1.X channels in Q-patch assays by increasing concentrations of the compound (N ≥ 4). Standard deviation is indicated by error bars.

Intraperitoneal administration (30 mg/kg) of compound **4** in mice resulted in sufficient brain exposure (193 ng/g 1 h after administration), which corresponded to 13 nM of free brain concentration[18] comparable to the *in vitro* potency of **4** (Table 6). This suggests that compound **4** has a potential use as an *in vivo* tool to investigate whether this type of Na$_v$1.1 activator can restore Nav1.1 functions and modify the disease state in animal models. Moreover, the multidrug resistance protein 1 (MDR1) membrane permeability of **4** (efflux ratio = 1.0) predicts that compound **4** can penetrate the blood–brain barrier (BBB) in humans.[19]

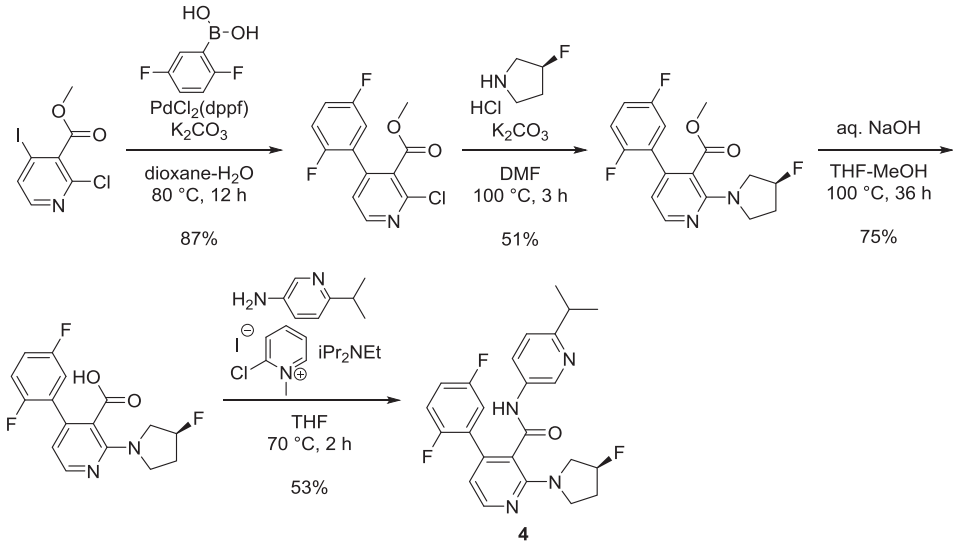
Finally, the subtype selectivity of **4** against Na$_v$1.2, Na$_v$1.3, Na$_v$1.5, and Na$_v$1.6 was evaluated in Q-patch assays (Fig. 2). While **4** activated Na$_v$1.3 to a lesser extent than Na$_v$1.1, it had no effect on Na$_v$1.2, Na$_v$1.5, or Na$_v$1.6 up to 10 μM.[20] The subtype selectivity of **4** was significantly improved compared with previously reported Na$_v$1.1 activators.

A typical synthetic route for the pyrimidine amide series is illustrated in Scheme 1 starting from commercially available methyl 4,6-dichloropyrimidine-5-carboxylate to provide representative compound **6**. A synthetic route for the nicotinamide derivative **4** from commercially available methyl 2-chloro-4-iodopyridine-3-carboxylate is demonstrated in Scheme 2. Further synthetic details are available in the Supporting Information.

The novel and potent Na$_v$1.1 activator **4** was designed and identified starting from the HTS hit **5**. Compound **4** showed sufficient brain exposure in mice, achieving comparable concentrations to its *in vitro* potency and exhibited improved subtype selectivity against Na$_v$1.X channels compared with previously reported Na$_v$1.1 activators. Therefore, compound **4** is a valuable Na$_v$1.1 activator for further evaluation of pathophysiological functions of the Na$_v$1.1 channel and has potential for therapeutic treatments of CNS diseases such as Dravet syndrome.



Scheme 1. Preparation of pyrimidine derivative 6.



Scheme 2. Preparation of nicotinamide derivative 4.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.01.023.

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