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Phenethyl nicotinamides, a novel class of $Na_V1.7$ channel blockers: Structure and activity relationship

Inger Kers [a,*], Istvan Macsari [a], Gabor Csjernyik [a], Martin Nylöf [a], Karin Skogholm [a], Lars Sandberg [a], Alexander Minidis [a], Tjerk Bueters [b], Jonas Malmborg [b], Anders B. Eriksson [c], Per-Eric Lund [d], Elisabet Venyike [d], Lei Luo [d], Jan-Erik Nyström [c], Yevgeni Besidski [a]

[a] Medicinal Chemistry, CNSP iMed Science, AstraZeneca R&D, Innovative Medicines, SE-15185 Södertälje, Sweden
[b] DMPK, CNSP iMed Science, AstraZeneca R&D, Innovative Medicines, SE-15185 Södertälje, Sweden
[c] CNSP iMed Project Management, AstraZeneca R&D, Innovative Medicines, SE-15185 Södertälje, Sweden
[d] Neuroscience, CNSP iMed Science, AstraZeneca R&D, Innovative Medicines, SE-15185 Södertälje, Sweden

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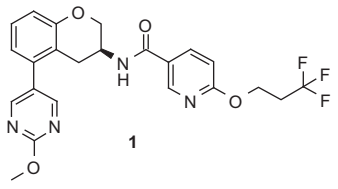
ABSTRACT

The $Na_V1.7$ ion channel is an attractive target for development of potential analgesic drugs based on strong genetic links between mutations in the gene coding for the channel protein and inheritable pain conditions. The (*S*)-*N*-chroman-3-ylcarboxamide series, exemplified by **1**, was used as a starting point for development of new channel blockers, resulting in the phenethyl nicotinamide series. The structure and activity relationship for this series was established and the metabolic issues of early analogues were addressed by appropriate substitutions. Compound **33** displayed acceptable overall in vitro properties and in vivo rat PK profile.

$Na_V1.7$ is a voltage-gated sodium channel with an important role in the progression of electrical signals in the nervous system caused by variations in membrane potentials of nerve cells.[1] This ion channel is an attractive target for development of potential analgesic drugs based on strong genetic links between mutations in the gene coding for the $Na_V1.7$ protein and inheritable pain conditions.[2] Hereditary loss of function mutations of $Na_V1.7$ in humans were reported to cause congenital inability to experience pain (CIP).[3,4] Importantly, although CIP is a severe condition by itself, it is not accompanied by other major physiological problems. In addition, gain of function mutations have been connected to the inheritable pain conditions erythromelalgia and familial rectal pain.[5] Tolerability issues limit the use of sodium channel blocker therapies that are currently available. Finding new compounds with increased potency for $Na_V1.7$ and higher subtype selectivity against other Na_V channels could greatly improve quality of life for a large population of patients suffering from pain.[6]

During the course of our $Na_V1.7$ channel blocker program we have established the structure-activity relationship in the (*S*)-*N*-chroman-3-ylcarboxamide series, represented by **1** (Fig. 1).[7] High potency on the $Na_V1.7$ channel and good functional selectivity over $Na_V1.5$, as measured in whole-cell voltage clamp electrophysiology assays using physiologically relevant activation protocols, were generally achieved within the series including compound **1**.[8,9] The $Na_V1.5$ channel is widely expressed in heart muscle and inhibition leads to ventricular arrhythmia. High selectivity against this subtype is therefore an important requirement for the progression of a potential drug candidate.[10,11] Close analogues to compound **1** showed low hERG activity, as measured in a whole-cell voltage-clamp electrophysiology assay.[12] In addition, this series displayed good in vitro metabolic stability in rat and human liver microsomes and showed no reactive metabolite formation. Compound **1** demonstrated low solubility however. We have found that this shortcoming can be addressed by modifying the heteroaromatic ring attached to the chromane in combination with the introduction of a methyl trifluoroethoxy side chain.



1

$Na_V1.7$ pIC_{50} 7.1
$Na_V1.5$ pIC_{50} 5.4
clogP 4.18
hERG pIC_{50} 4.9
Solubility 3 μM
RLM Clint <10 μL/min/μg
HLM Clint <10 μL/min/μg
GSH in HLM: -

Figure 1. Profile of chromane **1**.

* Corresponding author. Tel.: +46 8 553 28609.
E-mail address: inger.kers@astrazeneca.com (I. Kers).

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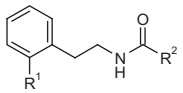
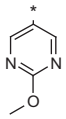
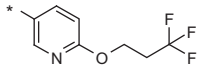
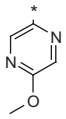
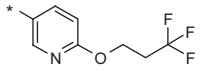
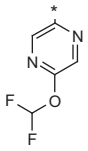
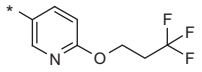
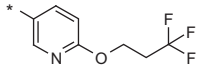
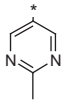
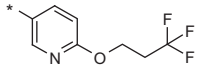
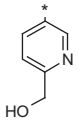
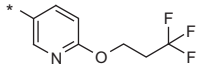
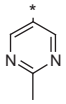
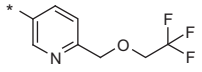
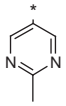
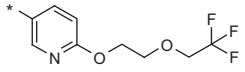
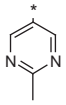
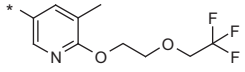
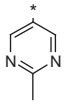
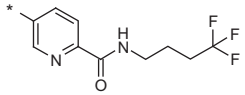
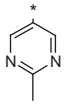
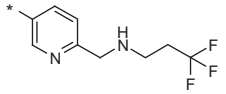
Table 1
SAR of unsubstituted phenethyl compounds **2**–**12**

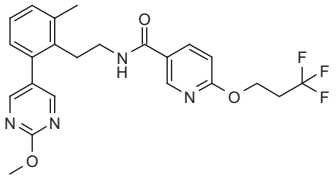
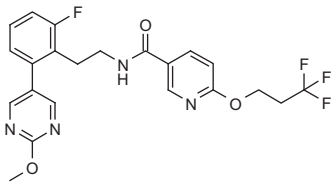
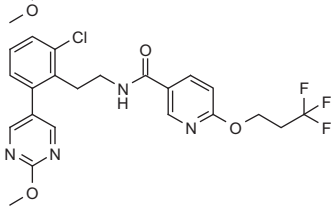
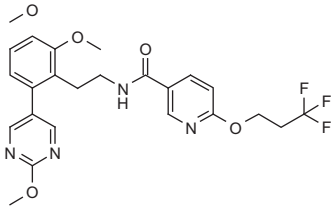
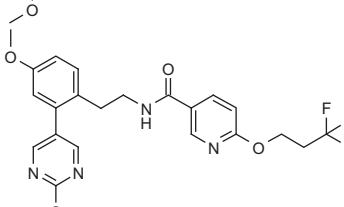
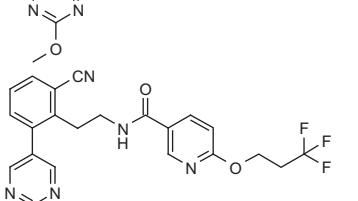
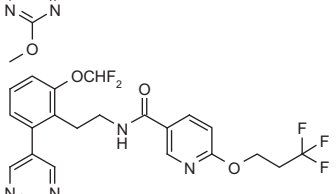
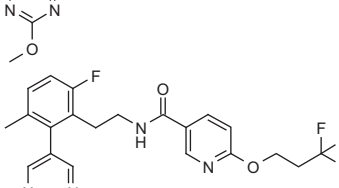
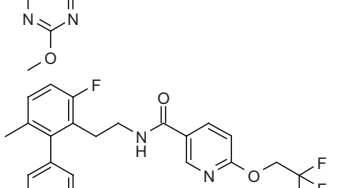
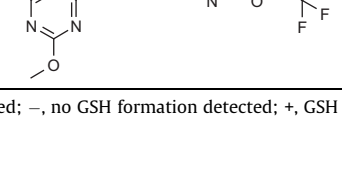
	R^1	R^2	c*Log*P	$Na_V1.7$ pIC_{50}	$Na_V1.5$ pIC_{50}	hERG pIC_{50}	Solubility (μM)	RLM/HLM Clint (μl/min/μg)	GSH trapping in HLM
1	–	–	4.18	7.1	5.4	4.9	3	<10/<10	–
2			3.87	6.9	4.9	5.0	57	>500/17	NM
3			4.08	7.6	5.2	4.7	12	129/124	++
4			4.52	7.6	5.2	5.2	4	270/210	NM
5			3.87	7.4	5.2	5.2	11	>500/160	++
6			3.42	7.2	5.3	4.9	35	>500/11	++
7			3.29	7.0	<4.9	<4.6	310	130/10	NM
8			2.29	5.7	<4.5	NM	376	<10/<10	++
9			3.19	6.4	<4.5	4.5	360	<10/23	NM
10			3.69	6.0	<4.5	NM	150	NM/NM	NM
11			2.76	6.6	5.2	4.7	89	<10/11	+++
12			1.98	5.5	<4.5	NM	390	<10/15	NM

NM, not measured; –, no GSH formation detected; +, GSH formation detected; ++, GSH formation abundant; +++, GSH formation extensive.

Core replacements of the chromane ring were limited to tetralin due to synthetic challenges. We were interested in exploring a different chemical space by opening up the chromane aliphatic ring as this would allow variation of the substituents on the resulting monocyclic core more easily. To this end a number of analogues were designed and prepared by varying the position and length of the linker between the phenyl core and the amido substituent. Generally, these analogues showed low affinity to the $Na_V1.7$ channel. Therefore we were pleased to see that in compound **2** (see Table 1) the $–OCH_2–$ fragment of the chromane ring could be removed without major influence on the $Na_V1.7$ potency. Structurally related to amidochromane **1**, compound **2** had a similar profile except for a diminished metabolic stability in rat liver microsomes and improved solubility. Encouraged by this finding we prepared close analogues to **2**, and the results are summarized in Table 1.

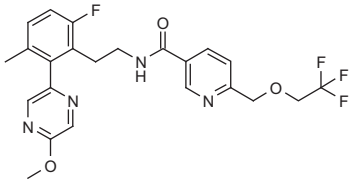
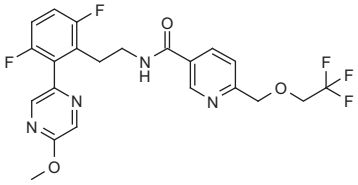
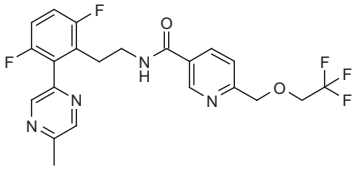
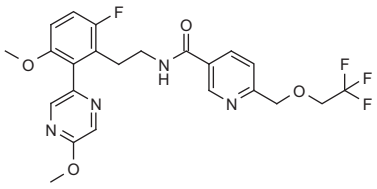
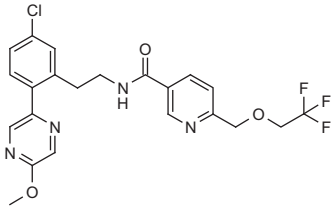
The replacement of the pyrimidine ring with pyrazine in **3** further increased the potency on $Na_V1.7$. In case of **4** the $Na_V1.7$ potency was retained (cf. **3**), but because *c Log P* was increased from 3.87 to 4.52 solubility and human metabolic stability were reduced compared to **2**. The use of unsubstituted pyridine (**5**), methylpyrimidine (**6**) and hydroxymethyl pyridine (**7**) rings also retained

Table 2
SAR of substituted phenethyl analogues **13–21**

Compound		cLogP	$Na_V1.7$ pIC_{50}	$Na_V1.5$ pIC_{50}	hERG pIC_{50}	Solubility (μM)	RLM/HLM Clint (μl/min/μg)	GSH trapping in HLM
1		4.18	7.1	5.4	4.9	3	<10/<10	–
13		4.32	7.5	5.2	5.2	2	32/65	–
14		4.01	7.2	5.5	5.1	30	13/<10	++
15		4.58	7.5	5.3	5.4	<4.5	<10/17	++
16		3.91	6.4	5.2	NM	15	17/45	NM
17		3.91	<5	4.7	5.2	2	150/40	NM
18		3.45	5.2	<4.5	NM	17	24/<10	–
19		4.36	<5	5.1	5.4	NM	190/<10	NM
20		4.21	7.5	6.1	NM	17	230/100	NM
21		4.23	7.6	5.6	4.9	40	35/74	–

NM, not measured; –, no GSH formation detected; +, GSH formation detected; ++, GSH formation abundant; +++, GSH formation extensive.

Table 3
SAR of substituted phenethyl analogues **22**–**26**

Compound		c*Log*P	$Na_V1.7$ pIC_{50}	$Na_V1.5$ pIC_{50}	hERG pIC_{50}	Solubility (μM)	RLM/HLM Clint (μl/min/μg)	GSH trapping in HLM
1		4.18	7.1	5.4	4.9	3	<10/<10	–
22		3.30	7.4	6.0	4.7	240	88/61	+
23		3.24	7.3	5.8	4.7	14	45/44	–
24		2.80	6.9	5.8	<4.5	120	<10/21	–
25		2.64	6.7	NM	NM	3	67/46	NM
26		3.67	<5.0	<4.5	NM	19	NM/72	NM

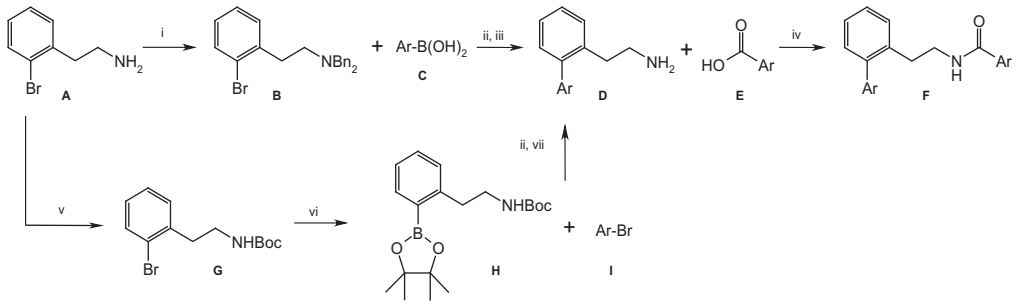
NM, not measured; –, no GSH formation detected; +, GSH formation detected; ++, GSH formation abundant; +++, GSH formation extensive.

the high $Na_V1.7$ potency whilst decreasing lipophilicity. Introduction of a homologous ether chain in **8** resulted in a substantial decrease in potency, cf. **6**. The chain elongation in compound **9** increased the potency (cf. **8**) but the introduction of a methyl group on the nicotinic acid moiety lowered it again (**10**, cf. **9**). Worth noticing is the unexpectedly high solubility for compounds **7** and **9**. Introduction of amido and amino groups in **11** and **12** gave only moderately potent compounds.

In general the structural changes of the heteroaromatic part of the molecules in **2**–**7** were well tolerated in terms of $Na_V1.7$ potency, but the resulting compounds were prone to high metabolic turnover particularly in rat liver microsomes. In contrast, **8, 9, 11** and **12** showed good metabolic stability, which may be partially due to reduced lipophilicity, but also suggested that metabolic stability, could be modulated by optimising the right-hand chain. The potency for $Na_V1.5$ and hERG was low throughout the sub-series and $Na_V1.7$ selectivity versus these channels was approximately 100 fold. In general, we observed that a decrease in c*Log*P was accompanied by an increase in solubility.

We also found evidence for the production of reactive metabolic intermediates using a glutathione trapping assay in cases of **3**, **5**, **6**, **8** and **11**.[13] Initial analysis of the biotransformation of these analogues suggested that glutathione adducts were localised on the core phenyl moiety. Further evidence of this was obtained from 6-fluorinated analogues, for which we observed that fluorine was replaced by hydroxyl (e.g. **14**, vide infra). The most plausible mechanism behind the reactive metabolite formation, based on this observation, is via an epoxidation of the phenyl ring.

In order to block the reactive metabolite formation and improve metabolic stability we focused our attention on substitutions on the phenyl core. The 6-position in the chromanes is blocked by an alkoxy moiety and therefore we started our investigation with introducing substituents in this position. The SAR data is shown in Table 2 and Table 3. Compound **13** was substituted with a methyl group which improved the metabolic stability in rat, but lowered it in human liver microsomes (cf. **2**). No glutathione adducts were observed. The incorporation of halogens such as fluorine in **14** and chlorine in **15** at the same position increased the stability, but failed to attenuate the reactive metabolite formation. On the other hand, the methoxy analogue (**16**) showed only moderate stability. In **17** the methoxy-moiety was placed in the 4-position which significantly decreased the metabolic stability in rats compared to that observed for compound **16**, indicating that the 6-position is important for maintaining stability, particularly in



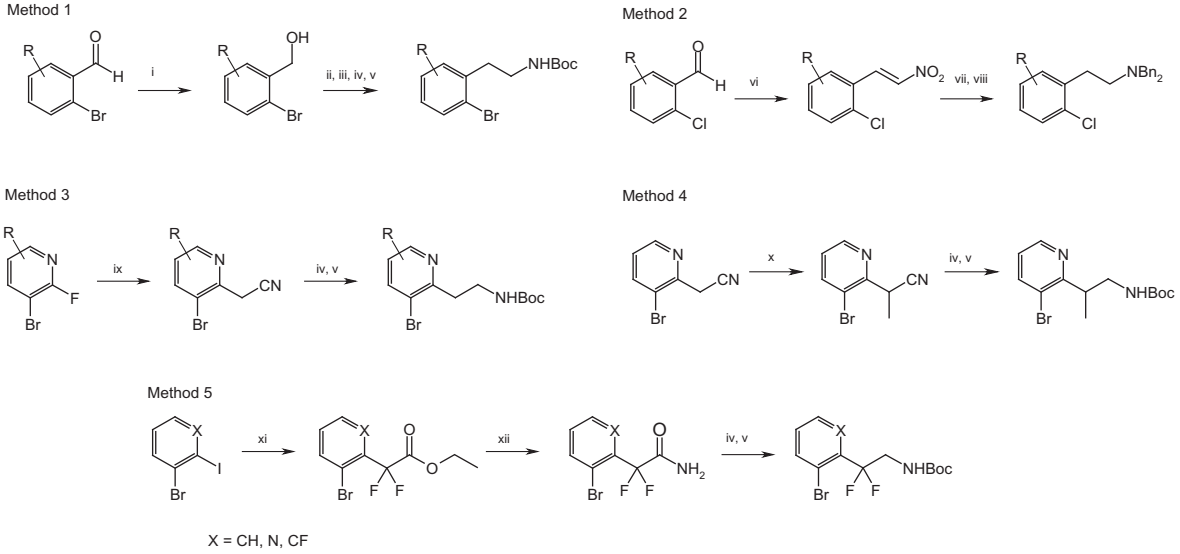
Scheme 1. Reagents and conditions: (i) Benzylbromide, K_2CO_3, KI, MeCN; (ii) $PdCl_2$·dppf, 2 M K_2CO_3, *i*PrOH, heating; (iii) 10% Pd/C, ammonium formate, MeOH, heating; (iv) HBTU, NEt_3, DMF or 1,1′-carbonyldiimidazole, EtOAc, 60 °C; (v) di-*tert*-butyl dicarbonate, NEt_3, DCM, 0 °C; (vi) bis(pinacolato)diboron, $PdCl_2$·dppf, KOAc, dioxane, heating; (vii) TFA, DCM.

rats. Substituents with strong electron withdrawing character gave moderately (**18**) and highly (**19**) unstable compounds in rats. Contrarily, the human intrinsic clearance was low for **18** and **19** which indicates that different metabolic pathways may be activated in rats compared to humans.

The fluorine atom at the 6-position improved the metabolic stability of **14** (cf. **2**), but addition of a methyl group at the 3-position as in **20** abolished the improvement in metabolic stability. An analogue to compound **20** with the shorter trifluoroethoxy substituent (**21**) showed a similar overall profile with the exception of clearly improved metabolic stability. In **22** the replacement of the pyrimidine ring with pyrazine and a homologous alkoxy side chain somewhat improved the stability (cf. **20**). Upon replacing the 3-methyl with fluorine in **23** the stability was somewhat improved. In case of **24** the replacement of the methoxy group on the pyrazine led to further increased metabolic stability of the compound. When the 3-methyl in **22** was replaced by a methoxy group (**25**) a marginally more stable compound was obtained. These 3- and 6-disubstituted analogues produced, however, only little or no reactive metabolites, which is an advantage over **14**. Chlorine at the 5-position in **26**, which was not substituted in the chromanes, did not have major influence on the metabolic stability.

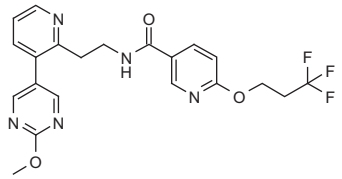
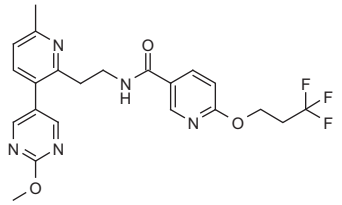
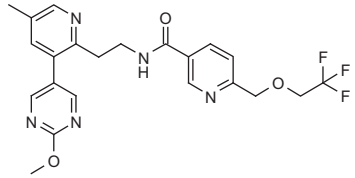
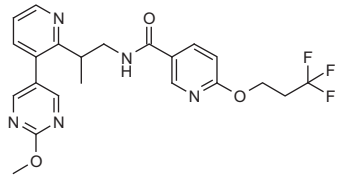
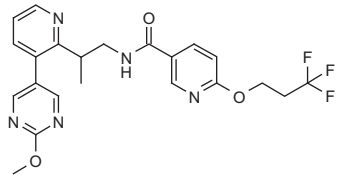
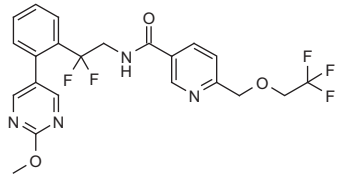
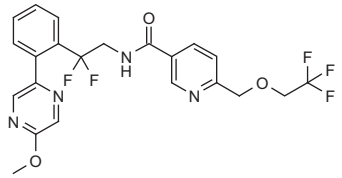
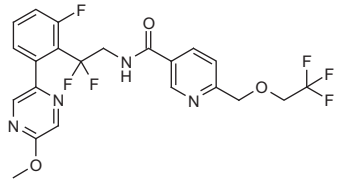
In general, the compounds with halogen or methyl substituents at the 6-position (**13**–**15**) exhibited high potency on $Na_V1.7$ channel, also with additional substituent in the 3-position (**20**–**25**). However, introducing substituents on the 4 or 5-positions afforded compounds **17** and **26** with no observed $Na_V1.7$ activity in the measured concentration range. Worth to notice is compound **16** with high apparent similarity to chromane **1**, but lower potency. Placing electron withdrawing substituents in 6-position substantially lowered the $Na_V1.7$ potency (compounds **18** and **19**). As we have previously seen for the unsubstituted analogues (Table 1) the low potency for $Na_V1.5$ and hERG was retained throughout the sub-series. The solubility was generally lower though, with exception of compound **22**. In conclusion, introduction of appropriate substituents on the phenyl core was found to improve metabolic stability and reduce formation of reactive metabolites while certain types of modifications also were well tolerated from a potency perspective.

The general synthesis of phenethyl nicotinamides is outlined in Scheme 1. The starting materials were either commercially available or could readily be prepared according to published methods.[14–17] Thus, the amino groups of **A** were dibenzylated and the formed **B** subsequently reacted with appropriate arylboronic acids **C**. Deprotection afforded **D** which were coupled with carboxylic acids **E**, yielding final products **F**. The commercial availability of heteroaryl boronic acids or esters (**C**) was limited and therefore we devised an alternative route to **D** which allowed the use of heteroaryl halides as coupling partners in the Suzuki reaction. Boc-protection of **A** afforded **G** which was converted to boronic ester **H** in a palladium promoted reaction. The formed intermediate **H** could subsequently be reacted with suitable arylbromides **I**,



Scheme 2. Reagents and conditions: (i) Sodium borohydride, THF; (ii) thionyl chloride, THF; (iii) NaCN, NEt_3, DMSO; (iv) BH_3-tetrahydrofuran complex, THF; (v) 10% NaOH, di-*tert*-butyl dicarbonate, THF; (vi) ammonium acetate, $MeNO_2$, heating; (vii) $LiAlH_4$, diethyl ether, 0 °C; (viii) benzylbromide, K_2CO_3, KI, MeCN; (ix) NaHMDS, MeCN, toluene, 0 °C; (x) MeI, NaH, DMF; (xi) ethyl bromodifluoroacetate, Cu bronze, DMSO, heating; (xii) NH_3 in methanol.

Table 4
SAR of compounds **27**–**34**

Compound		cLogP	$Na_V1.7$ pIC_{50}	$Na_V1.5$ pIC_{50}	hERG pIC_{50}	Solubility (μM)	RLM/HLM Clint (μl/min/μg)	GSH trapping in HLM
1		4.18	7.1	5.4	4.9	3	<10/<10	–
27		2.48	<5.0	<4.5	NM	360	<10/<10	–
28		2.98	<5.0	<4.5	NM	140	<10/<10	–
29		1.85	<5.0	<4.5	NM	260	<10/<10	NM
30		2.88	<5.0	<4.5	NM	370	<10/<10	NM
31		3.07	6.0	NM	NM	64	<10/<10	NM
32		3.34	6.2	4.7	4.7	190	13/<10	–
33		3.55	6.6	4.5	4.6	86	16/<10	–
34		3.69	6.7	5.0	4.7	52	24/<10	–

NM, not measured.; –, no GSH formation detected; +, GSH formation detected; ++, GSH formation abundant; +++, GSH formation extensive.

affording common intermediates **D**. Using the procedure described in Scheme 1 and starting from commercially available phenethylamines compounds **2**–**12**, **14** and **15** were prepared.

Many of the substituted phenethylamines were not commercially available and were prepared using one of the methods outlined in Scheme 2. The prepared phenethylamines of type **B** or **G** were then used to synthesise the desired compounds according to Scheme 1. Synthesis of the phenethylamines required for the preparation of compounds **18–24** and **26** is described in Method 1. For compounds **18** and **19** the starting materials were prepared according to literature procedures.[18,19] The synthesis of the phenethylamines required for compounds **13**, **16**, **17** and **25** is described

in Method 2. The phenethylamines used in the synthesis of compounds **27**–**29** is described in Method 3. For compound **30** synthesis of the required phenethylamine followed Method 4. For compounds **31**–**34** the synthesis of phenethylamines used is described in Method 5.

At this point of our investigation we decided to introduce a pyridine ring in the core, aiming to improve metabolic stability and to avoid reactive metabolite formation. Therefore close analogues to **2** were designed and prepared and their data is summarized in Table 4. Unfortunately compounds **27–30** did not show activity on the $Na_V1.7$ channel in the measured concentration range, probably in part due to their low lipophilicity, but showed high metabolic stability in both rat and human liver microsomes and reactive metabolites were absent. Compound **31**, that combine a pyridine core structure with a difluorinated ethylene linker, showed a moderate potency (cf. **27**–**30**) most likely due to increased c*Log*P in combination with finding a permitted region for introducing substituents.

The important finding that difluoro substitution on the ethylene linker is accepted from a potency perspective prompted us to turn our attention back to unsubstituted phenyl analogues, such as **2**, to further study this substitution with respect to metabolic stability. Therefore, compound **32** was prepared and tested (Table 4). We were particularly pleased to see that low intrinsic clearance in rats was measured suggesting acceptable pharmacokinetic properties in vivo in rat. For this series in general, the in vivo clearance could be predicted within a 2 fold based on the in vitro metabolic stability in rat. Furthermore, the compound retained moderate $Na_V1.7$ potency. The pyrazine analogue **33** and fluorine substituted **34** both showed further increased potency, similar metabolic profile and no reactive metabolite formation. In addition these compounds displayed good solubility and 30–100 fold selectivity over $Na_V1.5$ and hERG.In vivo rat PK data for compounds **1**, **13**, **22**, **23**, **24**, **32** and **33** is presented in Table 5.[20] The (*S*)-*N*-chroman-3-ylcarboxamide series generally displayed a low to moderate clearance and the bioavailability of the compounds could mainly be explained by the hepatic first-pass clearance. Compound **1** showed low clearance but a moderate bioavailability. When we opened up the chromane ring we initially found high clearance and consequently low bioavailability, exemplified by **13**. This impeded in vivo pharmacological testing of these initial analogues. The systemic clearance in rat was markedly improved by substituting the 3- and 6-positions of the central phenyl ring and/or by using a methyl trifluoroethyl ether chain as in **22**, **23** and **24**. In spite of this, the bioavailability remained less than 10%. Introduction of a difluoro substitution on the ethylene linker restored the bioavailability to that of the chromane series as shown by **32** and **33**.

Based on systemic clearance a much higher bioavailability was anticipated after oral administration (e.g. **22**, **23**). Potential contribution of intestinal metabolism in rat was dismissed using intestinal microsomes, rat S9 fraction and rat intestinal fluid. Per oral PK was also performed at a higher dose (**22** at 100 μmol/kg) but bioavailability remained unchanged, indicating that no saturable enzymes or transporters were involved. Oral administration of the non-selective CYP inhibitor 1-aminobenzotriazole (ABT) improved the bioavailability of compound **24** from 7% to 40%. This improvement correlated well with the estimated hepatic first-pass effect for compound **24** based on the systemic clearance, which was observed to decrease ~30% using ABT after intravenous administration.[21] We concluded from these combined results that the low bioavailability in the rat was not majorly due to metabolism, although first-pass metabolism did contribute. Subsequently, precipitation tests of **33** in fasted state simulated intestinal fluid (FaSSIF) using different formulations were performed. Precipitation of a fine particle suspension occurred immediately, which did not dissolve after 12 h. This suggested that the low bioavailability may be related to low solubility in physiological solutions.

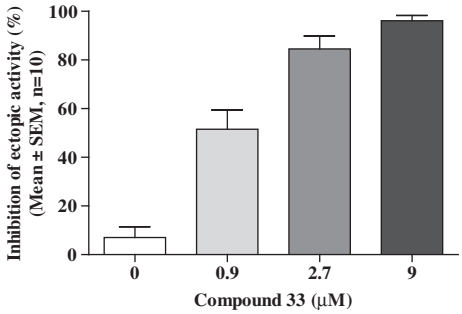


Figure 2. Concentration-dependent effects of compound **33** on ectopic firing from injured DRG neurons. Buffer or compound at 0.9, 2.7 and 9 μM were perfused cumulatively on DRGs excised from SNL-operated rats, 15 min for each concentration, and the effects during the last 3 min perfusion were analysed.

Low bioavailability mainly impeded in vivo pharmacological analysis of analogues in the described phenethyl nicotinamide series. Instead, efficacy of compound **33** was investigated in a rat DRG ex vivo preparation of SNL-induced ectopic activity.[22] Test concentrations of the compound was selected based on the in vitro potency for the rat $Na_V1.7$ channel; IC_{50} = 0.9 μM, using the same steady-state protocol (Vhold −65 mV) as for the human channel.[9] The average basal firing frequency of the tested fibres was 9.02 ± 1.86 Hz (mean ± SEM, *n* = 10). Compound **33** applied cumulatively on the DRGs caused progressive inhibition of ectopic firing of all fibres. At 0.9, 2.7 and 9 μM of compound **33** the ectopic activity was inhibited by 51.5 ± 7.9%, 84.5 ± 5.4% and 96.1 ± 2.2% (mean ± SEM), respectively (Fig. 2). Thus, the estimated EC_{50} for ex vivo inhibition of SNL-induced ectopic activity correlated very well to the in vitro $Na_V1.7$ potency of the compound. Similar correlation between in vitro $Na_v1.7$ potency and ex vivo inhibition of SNL-induced ectopic activity has been established for clinically used sodium channel blockers such as lidocaine, carbamazepine and lamotrigine (Kalezic et al., in preparation), as well as for a compound from a novel series of 3-Oxoisoindoline-1-carboxamides.[23]

In summary, we established the structure-activity relationship for a novel phenethyl nicotinamide series of $Na_V1.7$ blockers. We showed that metabolic issues could be addressed by introduction of appropriate substituents on the central phenyl ring and difluorination on the ethylene linker restored the bioavailability observed in the chromane series while retaining potency and selectivity versus $Na_V1.5$ and hERG at acceptable levels. As a result of our studies we found compound **33** that displayed good solubility and $Na_V1.7$ potency in addition to a 100 fold selectivity to hERG and $Na_V1.5$ channels. In contrast to earlier analogues in the phenethyl nicotinamide series compound **33** displayed good DMPK properties. Finally, we demonstrated efficacy in an ex vivo nerve

Table 5
Rat in vivo PK for compounds **1**, **13**, **22**, **23**, **24**, **32** and **33**

Compound	**1**	**13**	**22**	**23**	**24**	**32**	**33**
AUC/dose[a] (h kg/L)	1.2	0.33	0.91	1.3	0.68	0.71	1.5
V_{ss}[a] (L/kg)	7.2	2.4	1.5	0.85	1.74	1.4	0.72
$t_{½}$[a] (h)	6.4	0.98	0.90	0.90	5.9	2.0	1.2
CL[a] (mL/min/kg)	14	50	19	13	24.6	23	11
AUC/dose[b] (h kg/L)	0.32	0.002	0.03	0.06	0.05	0.24	0.41
C_{max}[b] (μmol/L)	0.23	0.02	0.53	0.16	0.25	0.84	1.4
t_{max}[b] (h)	4.8	0.25	1.5	0.67	0.50	0.75	0.33
$t_{½}$[b] (h)	7.6	2.4	3.6	3.0	2.3	2.4	2.8
F[b] (%)	26	0.6	4	5	7	34	27

[a] Intravenous (iv), dose 3 μmol/kg.
[b] Per oral (po), dose 10 μmol/kg.

injury model for compound **33** with good correlation to in vitro $Na_V1.7$ potency.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.08.031.

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