Bioorganic & Medicinal Chemistry Letters 22 (2012) 5618–5624

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Structure and activity relationship in the (S)-*N*-chroman-3-ylcarboxamide series of voltage-gated sodium channel blockers

Inger Kers [a,*], Gabor Csjernyik [a], Istvan Macsari [a], Martin Nylöf [a], Lars Sandberg [a], Karin Skogholm [a], Tjerk Bueters [b], Anders B. Eriksson [c], Sandra Oerther [d], Per-Eric Lund [d], Elisabet Venyike [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

ARTICLE INFO

Article history:
Received 7 June 2012
Revised 28 June 2012
Accepted 29 June 2012
Available online 7 July 2012

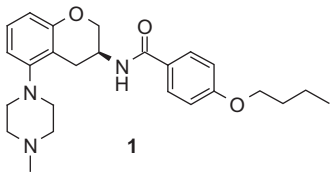
Keywords:
(S)-*N*-Chroman-3-ylcarboxamide
$Na_V1.7$ blocker
Neuropathic pain
Sodium channel blockers
Voltage-gated channel

ABSTRACT

Recent findings showing a relation between mutations in the $Na_V1.7$ channel in humans and altered pain sensation has contributed to increase the attractiveness of this ion channel as target for development of potential analgesics. Amido chromanes **1** and **2** were identified as blockers of the $Na_V1.7$ channel and analogues with modifications of the 5-substituent and the carboxamide part of the molecule were prepared to establish the structure–activity relationship. Compounds **13** and **29** with good overall in vitro and in vivo rat PK profile were identified. Furthermore, **29** showed in vivo efficacy in a nociceptive pain model.

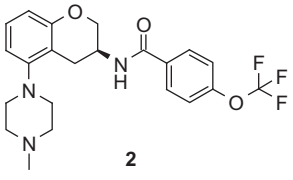
Voltage-gated sodium channels (VGSC) play a critical role in electrical signaling in the nervous system and are responsible for the initiation and propagation of action potentials caused by variations in membrane potentials.[1] Nerve signal transduction can be affected by altered rapid opening and closing of VGSCs and these channels are important targets in the pharmaceutical industry in regards to finding treatment for cardiac conductance disturbances, epilepsy and pain disorders.[2] Inherited loss of function mutations of $Na_V1.7$ in humans were reported to cause congenital inability to experience pain.[3,4] In addition, gain of function mutations have been connected to inheritable pain conditions erythromelalgia and familial rectal pain.[5] These observations make $Na_V1.7$ an attractive target for development of potential analgesics. Current sodium channel blocker therapies available have issues with tolerability and finding more potent compounds with higher subtype selectivity have potentially an important impact on treatment options for neuropathic pain.[6]

As a part of our preclinical $Na_V1.7$ channel blocker program aimed to discover novel analgesic drug candidates we examined a series of amido chromanes substituted at C-5 position. The initial hit series is represented by **1** and **2** (Fig. 1). The compounds were initially identified through HTS screening using a Li^+ flux atomic absorption spectroscopy assay.[7] Compound **1** showed moderate potency on $Na_V1.7$ and moderate selectivity over $Na_V1.5$, as measured in a whole-cell voltage clamp electrophysiology assays.[8,9] The $Na_V1.5$ channel is widely expressed in heart muscle and inhibition leads to ventricular arrhythmia, therefore very high



1

$Na_V1.7$ pIC_{50} 6.0
$Na_V1.5$ pIC_{50} <5.2
clogP 4.64
hERG pIC_{50} 5.2
Solubility 1 μM
RLM Clint 82 μL/min/μg
HLM Clint 361 μL/min/μg



2

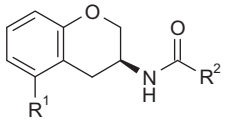
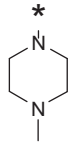
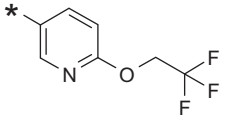
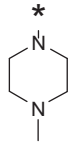
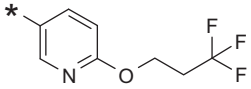
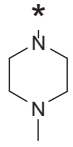
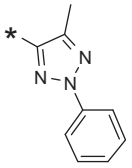
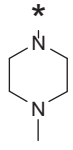
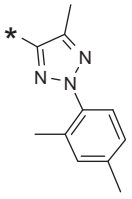
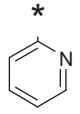
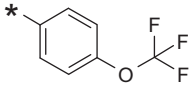
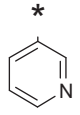
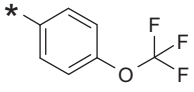
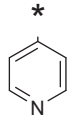
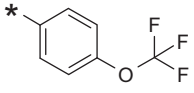
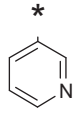
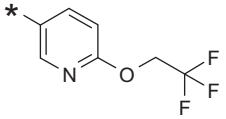
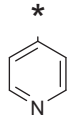
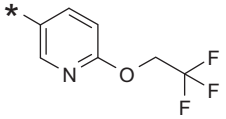
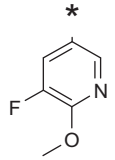
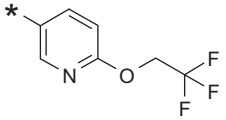
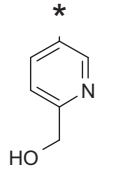
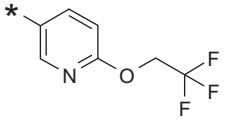
$Na_V1.7$ pIC_{50} 5.7
$Na_V1.5$ pIC_{50} 5.7
clogP 4.2
hERG pIC_{50} 5.1
Solubility 27 μM
RLM Clint 18 μL/min/μg
HLM Clint 2.5 μL/min/μg

Figure 1. Profile of initial hits, chromanes **1** and **2**.

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

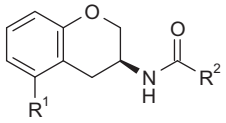
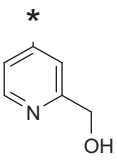
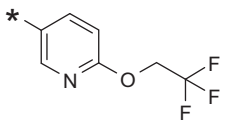
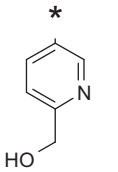
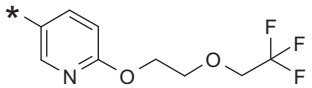
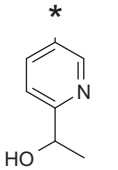
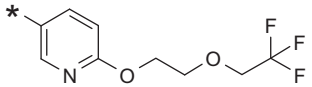
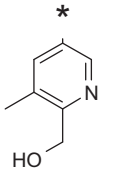
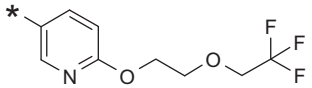
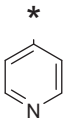
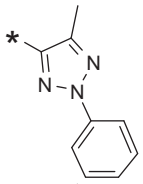
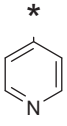
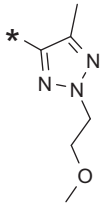
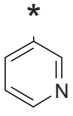
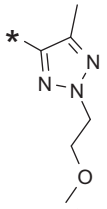
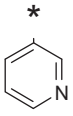
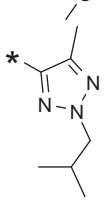
0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.06.105

Table 1
Chromanes with piperazine or pyridine as R^1 substituent

	R^1	R^2	NaV1.7 pIC_{50}	NaV1.5 pIC_{50}	hERG pIC_{50}	c*Log*P	Solubility (μM)	RLM/HLM Clint (μl/min/μg)
3			6.1	5.3	5.1	3.82	82	<10/<10
4			6.2	5.3	4.9	3.80	379	<10/<10
5			6.5	5.3	5.1	3.53	211	4/14
6			6.8	5.6	5.0	4.53	4	9/stable
7			5.8	ND[a]	ND[a]	4.86	ND[a]	ND[a]
8			5.9	5.5	ND[a]	4.65	5	ND[a]
9			6.	5.4	5.6	4.65	2	ND[a]/78
10			7.1	5.5	4.6	4.19	29	64/161
11			7.0	5.5	5.2	4.19	9	77/59
12			6.4	5.4	4.6	4.99	1.1	10/19
13			6.8	5.2	5.0	3.60	58	<10/10

(*continued on next page*)

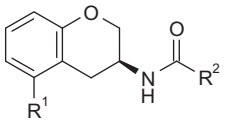
Table 1 (*continued*)

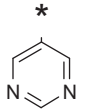
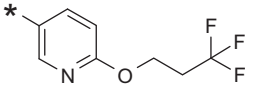
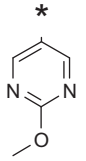
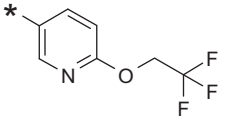
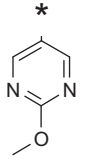
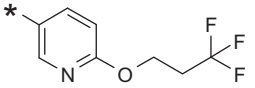
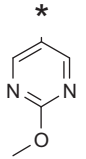
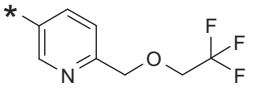
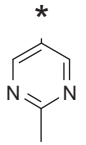
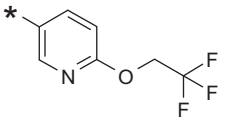
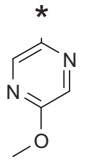
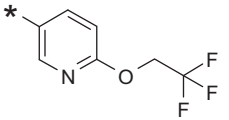
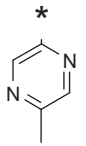
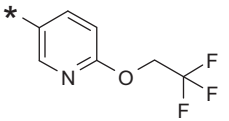
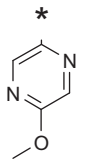
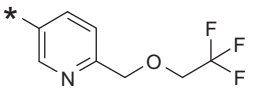
			NaV1.7 pIC_{50}	NaV1.5 pIC_{50}	hERG pIC_{50}	c*Log*P	Solubility (μM)	RLM/HLM Clint (μl/min/μg)
14			6.3	5.2	4.8	3.60	17	<10/15
15			6.8	5.1	4.8	3.35	54	<10/11
16			6.1	5.0	ND[a]	3.21	18	<10/12
17			6.1	5.3	ND[a]	3.80	18	ND[a]
18			5.9	<4.5	5.4	3.90	2	ND[a]
19			4.6	<4.5	ND[a]	1.67	306	ND[a]
20			5.5	<4.5	ND[a]	1.67	396	ND[a]
21			5.4	<4.5	ND[a]	3.08	393	ND[a]

[a] ND: not determined.

selectivity against this subtype is an absolute requirement for the progression of a compound.[10,11] Moreover, **1** showed moderate hERG activity, as measured in a whole-cell voltage-clamp electrophysiology assay.[12] Compound **1** displayed poor solubility and low metabolic stability. In contrast, **2** exhibited medium solubility and improved metabolic stability, but was slightly less potent in

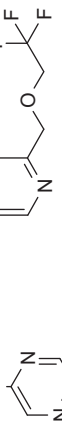
Table 2
SAR of chromanes with diazine as R^1 substituent



	R^1	R^1	NaV1.7 pIC50	NaV1.5 pIC_{50}	hERG pIC_{50}	c *Log P*	Solubility (μM)	RLM/HLM clint (μl/min/μg) 22
22			7.2	5.4	5.2	3.24	23	38/318
23			7.1	5.2	5.1	4.20	<1	<10/<10
24			7.1	5.4	4.9	4.18	3	<10/<10
25			6.6	5.0.	4.8	3.06	145	<10/<10
26			7.0	5.3	5.0	3.76	6.5	<10/<10
27			6.9	<4.6	<4.5	4.41	<1	14/14
28			7.1	<5.5	<4.5	3.97	<1	<10/16
29			7.1	4.9	4.9	3.27	34	<10/<10

(*continued on next page*)

Table 2 (*continued*)

			NaV1.7 pIC50	NaV1.5 pIC_{50}	hERG pIC_{50}	c *Log P*	Solubility (μM)	RLM/HLM clint (μl/min/μg) 22
30			6.3	4.7	ND[a]	2.82	82	<10/<10
31			<5.0	ND[a]	ND[a]	3.27	ND[a]	21/16

[a] ND: not determined.

blocking $Na_V1.7$ and did not show selectivity against the $Na_V1.5$ channel. Chromanes with basic substituents are reported to block 5-HT receptors and, not surprisingly, both compounds were proven to be potent antagonist of the 5-HT_{1A} and 5-HT_{1B} receptors.[13–15]

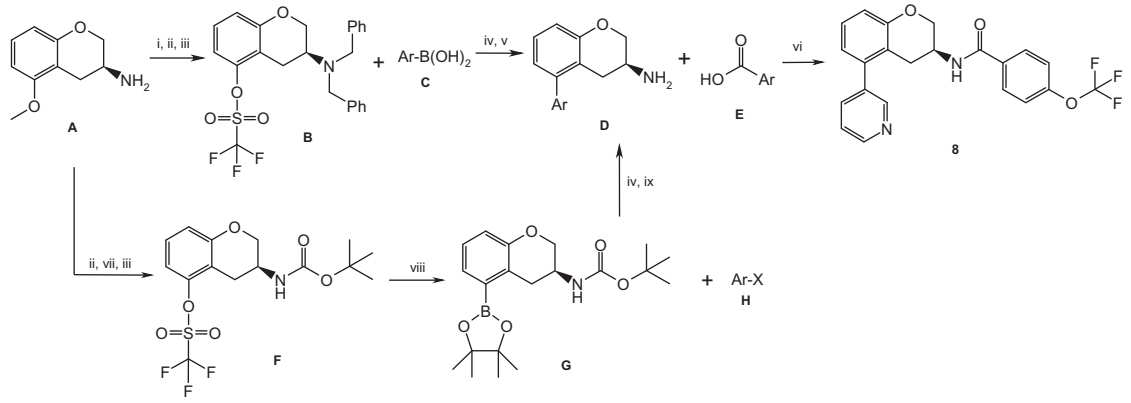
The early findings mentioned above have prompted us to conduct further investigations. First, we probed the effect of lowering the lipophilicity on the $Na_V1.7$ potency by introducing more polar acyl groups than those represented in **1** and **2**. Therefore the phenyl ring was replaced with a pyridine ring (**3** and **4,** Table 1) which yielded less lipophilic, consequently more soluble, compounds that retained the moderate $Na_V1.7$ potency (cf. **1**). In case of **5** a phenyl substituted triazole ring was tried as bioisosteric acyl group. This modification also proved to be successful since compound **5** exhibited increased potency, demonstrated excellent solubility and high metabolic stability. When methyl groups were introduced on the phenyl ring in **6**, a more potent $Na_V1.7$ blocker was obtained, however the $Na_V1.5$ activity was also increased and the solubility dramatically decreased.

In general compound **5** exhibited a good overall profile, but unfortunately potency on the 5-HT receptors was still high which confirmed that this undesired affinity was caused by the presence of a basic piperazino moiety. We reasoned that by decreasing the basicity of the target molecules the potential 5-HT liability could be eliminated. Thus, we focused our attention on basic heterocyclic rings to find adequate replacements for the piperazino substituent. Compounds **7**–**9** with isomeric pyridines as a 5-substituent were prepared and we found that 2- and 3-pyridyls (**7** and **8**) were equipotent with the 5-methylpiperazinyl analogue (cf. **2**) while the 4-pyridyl **9** exhibited markedly higher $Na_V1.7$ potency. However, these highly lipophilic compounds (**7**–**9**) were poorly soluble. Therefore we decided to address this issue by combining the two above mentioned successful modifications, the introduction of pyridine rings at both C-5 and in the acyl moiety. Indeed, compounds **10** and **11** showed not only high potency and improved selectivity versus $Na_V1.5$ but were more soluble as well. Unfortunately, those gains were accompanied by low metabolic stability. We believed that unsubstituted positions of the pyridine ring potentially were affected in the metabolic process and decided to introduce substituents at both *meta* and *para* positions of the pyridine ring. We were pleased to see that the metabolic stability of compound **12** significantly improved, unfortunately, potency and solubility were impacted in an undesirable way.

Since good metabolic stability could be combined with the desired structural feature of a substituted pyridine as a substituent on the chromane ring, we continued our studies with the introduction of the polar hydroxymethyl group onto the pyridine ring (**13** and **14**). The 3-pyridyl isomer (**13**) displayed higher solubility and was markedly more potent than the 4-pyridyl **14**, while they were equipotent on the $Na_V1.5$ channel. In addition, both **13** and **14** exhibited excellent metabolic stability and, more importantly, the 5-HT activity was absent. Even when a longer alkoxy chain was introduced on the acyl fragment as in case of **15** the good potency, subtype selectivity, metabolic stability and solubility were still retained, cf. **13**. However, further substitution at the 5-pyridyl group, both branching in the substituent (**16**) or disubstitution (**17**) proved to be unfavorable for potency and solubility.

In parallel to the development of the dipyridyl chromane amides we investigated a subclass of compounds with pyridine as 5-substituent and triazole as a core group in the acyl part of the molecule (**18–21**). Theses analogues of **5** with weakly basic C-5 substituents, in contrast to dipyridine sub-series (like **11**), showed surprisingly low potency against $Na_V1.7$. In addition, the potency on the $Na_V1.5$ channel was also low.

As a result of the studies described above we considered dipyridyl chromane amide **13** a promising candidate for further characterization based on the good overall profile of the compound



Scheme 1. Reagents and conditions: (i) Benzylbromide, K_2CO_3, CH_2Cl_2; (ii) BBr_3, CH_2Cl_2, −78 °C; (iii) trifluoromethanesulfonic anhydride, NEt_3, CH_2Cl_2, 0 °C; (iv) $PdCl_2$.dppf, 2 M K_2CO_3, DMF, heating; (v) 10% Pd/C, ammonium formate, EtOH, heating; (vi) HBTU, NEt_3, DMF; (vii) di-tert-butyl dicarbonate, NEt_3, CH_2Cl_2, 0 °C; (viii) bis(pinacolato)diboron, $PdCl_2$·dppf, KOAc, dioxane, heating; (ix) TFA, CH_2Cl_2.

Table 3
Rat in vivo PK for compounds **13** and **29**

Compound	**13**	**29**
AUC/dose[a] (h∗kg/L)	0.40	0.6
V_{ss}[a] (L/kg)	11	4.2
$t_{½}$[a] (h)	3.8	2.2
CL[a] (mL/min/kg)	42.2	25
AUC/dose[b] (h∗kg/L)	0.27	0.3
C_{max}[b] (μmol/L)	0.38	0.30
t_{max}[b] (h)	1.5	2.5
$t_{½}$[b] (h)	3.5	4.8
F[b] (%)	66.3	44

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

(*vide infra*). However, we were still interested to see how the pyridine ring replacement with other heteroaryl groups at the 5-position effects the SAR. The data of the synthesised compounds is summarized in Table 2.

First, an unsubstituted pyrimidine ring was introduced in **22** which showed as high $Na_V1.7$ blocking activity and poor metabolic stability as **10**. The introduction of a methoxy substituent on the 4-position of the pyrimidine ring (**23** and **24**) led to retained good $Na_V1.7$ potency and successfully improved metabolic stability. Unfortunately, the solubility decreased substantially. On the positive note, compound **25** exhibited a markedly improved solubility while the lower lipophilicity was accompanied by a slight decrease

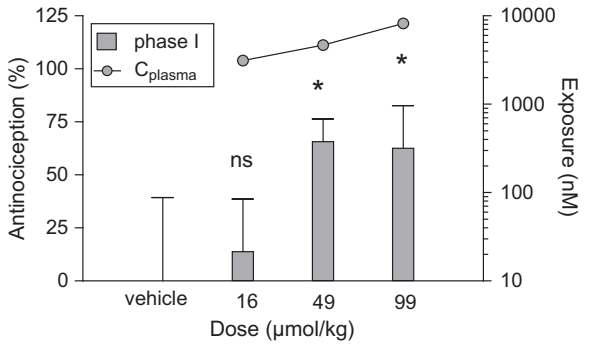


Figure 2. Dose–response effect of **29** in phase 1 of the rat formalin model after p.o. administration. The effect (histogram) is expressed as the percentage in comparison to vehicle treated group and total plasma concentration (line) is expressed as mean ± SD, n = 9 per group). One Way ANOVA was used as statistic in comparison to vehicle group per phase, followed by unpaired one-tailed t-test.*p <0.05.

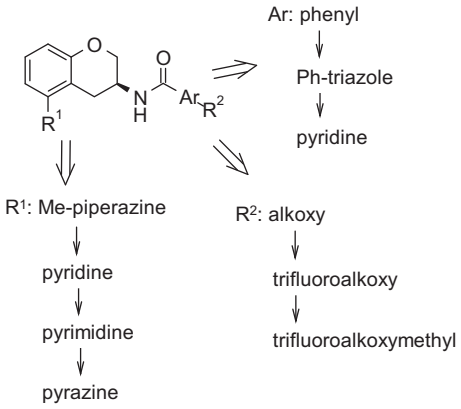


Figure 3. SAR of amido chromanes.

of potency (cf. **24**). As was observed for methoxy pyrimidine **23** the metabolism could also be retarded by introduction of a methyl group on the pyrimidine ring (**26**) but, again, this modification was detrimental for solubility.

We have also introduced 4-methoxy pyrazine (**27**) as aryl substituent on the chromane ring. This compound was a potent blocker of the $Na_V1.7$ channel and exhibited good selectivity versus $Na_V1.5$ but was poorly soluble. Replacement of the 4-methoxy group with a methyl moiety in **28** also afforded a potent but poorly soluble compound (cf. **26**). On the positive note both **29** and **30** proved to be more soluble (cf. **27** and **28**). Unfortunately, only **29** retained the high $Na_V1.7$ potency and good selectivity over $Na_V1.5$.

The stereochemistry of the amido group seemed to be very important to achieve high potency in the series. In order to verify this significance the corresponding R-enantiomer of **29**, compound **31**, was also prepared. We have found that the R-enantiomer did not exhibit $Na_V1.7$ channel blocking activity in the measured concentration range.

The general synthesis of amido chromanes is outlined in Scheme 1. The starting materials were either commercially available or could readily be prepared according to published methods.[16–18] Aminochromane **A** was benzyl protected and then the methyl group was removed to furnish a phenolic intermediate which was transformed to **B** by reaction with trifluoromethanesulfonic anhydride. Thereafter **B** was subjected to Suzuki reaction with the corresponding aryl boronic acid **C** followed by deprotection to give aminochromane **D**. The final product, represented by **8**, was obtained by coupling **D** with the appropriate acid **E**.

The commercial availability of heteroaryl boronic acids or esters (**C**) is limited and therefore we devised an alternative route to **D** which allowed the use of heteroaryl halides as coupling partners in the Suzuki reaction. The methyl group of **A** was removed and the resulting phenolic intermediate was converted to **F** in a one-pot procedure where the amino group was BOC protected followed by introduction of the triflate moiety. Thereafter **F** was converted in a palladium promoted reaction to boronic ester **G** which after a Suzuki reaction with aryl halide **H** and subsequent deprotection provided aminochromane **D**.

Compounds **13** and **29** presented attractive overall profiles in terms of high $Na_V1.7$ potency and selectivity versus $Na_V1.5$ and hERG, low lipophilicity, good solubility and high in vitro metabolic stability. A more comprehensive in vitro profiling, in enzyme and radioligand binding assays, of **13** and **29** was conducted on a panel of 98 different targets at MDS pharma. At 10 μM concentration compound **13** showed 55% inhibition at the Adrenergic α_{2B} receptor with [^{3}H] Rauwolscine as radioligand, 80% inhibition at the Dopamine Transporter (DAT) with [^{125}I] RTI-55 as radioligand and 98% inhibition of [^{125}I] Ghrelin at the Growth Hormone Secretagogue (GHS) receptor. Subsequently, IC_{50} values for **13** were determined to 2 μM for DAT and 0.2 μM for the GHS receptor. At 10 μM compound **29** showed 66% inhibition at the Adenosine Transporter (AT) with [^{3}H] Nitrobenzylthioinosine as radioligand, 55% inhibition at the Cannabinoid B1 (CB1) receptor with [^{3}H] SR141716A as radioligand and 127% increased binding of [^{125}I] Macrophage Inflammatory Protein-1α (MIP-1α to the CC Chemokine Receptor 1 (CCR1). The IC_{50} values for **29** were determined to 1.8 μM for AT and 5 μM for the CB1 receptor. Importantly, compound 29 was inactive when analysed for agonistic properties at the CB1 receptor in a GTP[γ][^{35}S] incorporation assay. In addition, both compounds were inactive in the $5HT_{1A}$ and $5\text{-}HT_{1B}$ assays.

Rat in vivo pharmacokinetics (PK) measurements were performed for **13** and **29** and the results are summarized in Table 3.[19] The similar in vitro metabolic stability of **13** and **29** is reflected well in their in vivo PK profile, particularly long half life and high oral bioavailability. In vivo efficacy of **29** was studied in the rat formalin model, which is a peripherally driven model with components of central sensitization.[20–22] As shown in Figure 2, the compound displayed a dose dependent antinociceptive effect in the phase 1 of the formalin test. A statistically significant antinociceptive effect of 66% was detected at a dose of 49 μmol/kg, corresponding to a total plasma concentration of 5 ± 1 μM. No significant antinociceptive effects were seen in phase 2 (data not shown).

In summary, we established the structure-activity relationship in the 5-heteroaryl 3-amido chromane class of $Na_V1.7$ blockers, see Figure 3 for summary of key-steps in optimization process. We started from the moderately potent but poorly soluble and metabolically unstable methylpiperazines (**1–6**) which also possessed 5-HT activity. We have shown that the combination of the less basic pyridine (**7**–**21**) and diazine rings (**22**–**30**) on the chromane ring with alkoxy pyridine acyl group could give more potent and subtype selective compounds which neither showed activity on the $5\text{-}HT_{1A}$ or $5HT_{1B}$ receptors, nor on the hERG channel. Compounds **13** and **29** showed the best overall in vitro profile, that translated well to rat in vivo PK. Furthermore, compound **29** showed significantly dose-dependent efficacy in the phase 1 of the formalin model of pain, whereas no effects were seen in phase 2.

Acknowledgments

The authors wish to thank colleagues at Physical Chemistry Characterization Team for providing solubility data, and the Safety Screening Centre for providing $Na_V1.5$ and hERG data. In addition, Dr. Alexander Minidis is acknowledged for fruitful discussions.

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.105.

References and notes

1. Sulayman, D. D. H.; Cummins, T. R.; Black, J. A.; Waxman, S. G. *Annu. Rev. Neurosci.* **2010**, *33*, 325.
2. Chahine, M.; Chatelier, A.; Babich, O.; Krupp, J. J. *CNS Neurol. Disord. - Drug Targ.* **2008**, *7*, 144.
3. Cox, J. J.; Reimann, F.; Nicholas, A. K.; Thornton, G.; Roberts, E.; Springell, K.; Karbani, G.; Jafri, H.; Mannan, J.; Raashid, Y.; Al Gazali, L.; Hamamy, H.; Valente, E. M.; Gorman, S.; Williams, R.; Mchale, D. P.; Wood, J. N.; Gribble, F. M.; Woods, C. G. *Nature* **2006**, *444*, 894.
4. Ahmad, S.; Dahllund, L.; Eriksson, A. B.; Hellgren, D.; Karlsson, U.; Lund, P-E.; Meijer, I. A.; Meury, L.; Mills, T.; Moody, A.; Morinville, A.; Morten, J.; O'Donnell, D.; Raynoschek, C.; Salter, H.; Rouleau, G. A.; Krupp, J. J. *Hum. Mol. Genet.* **2007**, *16*, 2114.
5. Yang, Y.; Wang, Y.; Li, S.; Xu, Z.; Li, H.; Ma, L.; Fan, J.; Bu, D.; Liu, B.; Fan, Z.; Wu, G.; Jin, J.; Ding, B.; Zhu, X.; Shen, Y. *J. Med. Genet.* **2004**, *41*, 171.
6. Zuliani, V.; Rivara, M.; Fantini, M.; Constantino, G. *Expert Opin. Ther. Patients* **2010**, *20*, 755.
7. Trivedi, S.; Dekermendjian, K.; Julien, R.; Huang, J.; Lund, P. E.; Krupp, J.; Kronqvist, R.; Larsson, O.; Bostwick, R. *Assay Drug Dev. Technol.* **2008**, *6*, 167.
8. Harmer, A. R.; Abi-Gerges, N.; Easter, A.; Woods, A.; Lawrence, C. L.; Small, B. G.; Valentin, J.-P.; Pollard, C. E. *J. Pharm. Tox Methods* **2008**, *57*, 30.
9. $Na_V1.7$ currents were recorded from HEK293cells stably expressing the α-subunit of the human channel (Millipore) with the perforated whole-cell configuration using IonWorks™ Quattro planar patch clamp automated electrophysiology system (Molecular Devices, Inc.). The external recording solution (D-PBS, Gibco) contained (in mM) NaCl (138), KCl (2.7), KH_2PO_4 (1.5), Na_2HPO_4 (8), $CaCl_2{\cdot}2H_2O$ (0.9) and $MgCl_2{\cdot}6H_2O$ (0.5) and glucose (5.5). The internal recording solution contained (in mM) *K*-gluconate (100), KCl (40), $MgCl_2$ (3), EGTA (3) and HEPES (5). KOH was used adjust pH to 7.25, and the final osmolarity was set at 290 mOsm. Amphotericin B was used at a final concentration of 150 μg/ml. A steady-state protocol was used where the cells were clamped to −65 mV and the sodium currents were evoked by a voltage train consisting of eight 30 ms depolarizing steps to −20 mV, at a frequency of 3 Hz. In order to recover a fraction of the channels from slow inactivation a 20 ms hyperpolarizing step to −100 mV was executed prior to the activation step. The fraction of inactivated channels in the assay was estimated to be 40−50%. The protocol was activated twice, pre-scan and post-scan compound addition and the degree of inhibition for each well was assessed for the eight pulse. Half log serial dilutions of the compounds were prepared in DMSO, with a concentration in the external recording solution of 0.3%.
10. Goldin, A. L. *Annu. Rev. Physiol.* **2001**, *63*, 871.
11. Tfelt-Hansen, J.; Winkel, B. G.; Grunnet, M.; Jespersen, T. *J. Cardiov. Electrophysiol.* **2010**, *21*, 107.
12. Bridgland-Taylor, M. H.; Hargreaves, A. C.; Easter, A.; Orme, A.; Henthorn, D. C.; Ding, M.; Davis, A. M.; Small, B. G.; Heapy, C. G.; Abi-Gerges, N.; Persson, F.; Jacobson, I.; Sullivan, M.; Albertson, N.; Hammond, T. G.; Sullivan, E.; Valentin, J. P.; Pollard, C. E. *J. Pharm. Tox Methods* **2006**, *54*, 189.
13. Berg, S.; Linderberg, M.; Ross, S.; Thorberg, S-O.; Ulff, B. WO 9914213, *1999*; *PCT Int. Appl.*,
14. Preparation of compounds 1 and 2: Berg, S.; Nylöf, M.; Ross, S.; Thorberg, S-O. WO 9914212 A1 19990325**, 1999**. *PCT Int. Appl.*
15. Ahlgren, C.; Eriksson, A.; Tellefors, P.; Ross, S. B.; Stenfors, C.; Malmberg, Å. *Eur. J. Pharmacol.* **2004**, *499*, 67.
16. Preparation of compounds **7**–**13**, **15**, **18**, **20**–**26**: Besidski, Y.; Kers, I.; Nylöf, M.; Sandberg, L.; Skogholm, K. WO2008130320, 2008, *PCT Int. Appl.*
17. Preparation of compounds **5** and **6**: Besidski, Y.; Kers, I.; Macsari, I.; Nylöf, M.; Rotticci, D. WO2008130321, 2008, *PCT Int. Appl.*
18. Preparation of compound 29: Aurell, C-J.; Besidski, Y.; Kers, I.; Nylöf, M. WO2012039657, 2012; *PCT Int. Appl.*
19. Formulation of compound **13** for both iv and po studies: 5% dimethylamine (DMA), 95% hydroxypropyl-β-cyclodextrine (HPbCD) (200 mg/ml). In case of **29** 3% DMA, 10% HPbCD (100 mg/ml), 2.35% glycerol were used for iv studies and 5% DMA, 10% HPbCD (100 mg/ml), 2.35% glycerol for po studies. In all cases fasted, Sprague–Dawley, male rats were used, Three animals/administration/compound.
20. The formalin test was performed by injecting 100 μL of 2.0% formalin subcutaneously on the dorsal side of the left hind paw of Sprague Dawley rats (n = 9 per group). Three hours prior to the formalin injection, compound **29**, at doses 0, 19, 49 and 99 μmol/kg, was administered per oral gavage. The behavior of the animals was recorded for 35 minutes after formalin injection and time spent to licking the injected paw was analyzed to represent nociceptive behavior. The response to formalin injection is biphasic; phase 1 (0–5 min after formalin injection) and phase 2 (15–35 min after formalin injection). Formulation: nanosuspension done of 1% PVP K30 and 0.2% SDS.
21. Gustafsson, H.; Sandin, J. *Eur. J. Pharmacol.* **2009**, *605*, 103.
22. Tjolsen, A.; Berge, O. G.; Hunskaar, S.; Rosland, J. H.; Hole, K. *Pain* **1992**, *51*, 5.