Bioorganic & Medicinal Chemistry Letters 21 (2011) 5197–5201

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of a novel series of selective HCN1 blockers

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ARTICLE INFO

Article history:
Received 6 May 2011
Revised 11 July 2011
Accepted 13 July 2011
Available online 23 July 2011

Keywords:
HCN1
HCN4
Ion channel
VIPR assay
Strecker reaction
Spared nerve injury model
Analgesic
Bradycardia
Neuropathic pain

ABSTRACT

The discovery of a series of novel, potent, and selective blockers of the cyclic nucleotide-modulated channel HCN1 is disclosed. Here we report an SAR study around a series of selective blockers of the HCN1 channel. Utilization of a high-throughput VIPR assay led to the identification of a novel series of 2,2-disubstituted indane derivatives, which had moderate selectivity and potency at HCN1. Optimization of this hit led to the identification of the potent, 1,1-disubstituted cyclohexane HCN1 blocker, 2-ethoxy-*N*-((1-(4-isopropylpiperazin-1-yl)cyclohexyl)methyl)benzamide. The work leading to the discovery of this compound is described herein.

Patients suffering from peripheral nerve damage frequently experience a variety of painful, spontaneous and evoked sensations. These sensations include sensitivity to light touch (tactile allodynia), pressure and thermal stimuli. Discharges emanating from both the site of injury and the dorsal root ganglion (DRG) are now believed to be responsible for the initiation and maintenance of neuropathic pain syndromes.[1] Recent findings suggest that a family of hyperpolarization-activated, cation-nonselective, cyclic nucleotide-modulated (HCN) channels contributes to the abnormal spontaneous discharges emanating from the DRG of damaged nerves and the increased firing rate with evoked stimuli.[1,3,4] Because HCN1 is expressed at much higher levels than the other subtypes in DRG neurons,[2] this channel has been proposed as a novel target for pharmacological intervention of pain.[1] A recent review discusses other clinical indications and potential side effects of HCN inhibitors.[15]

Most of the HCN inhibitors reported in the literature are equipotent at the four HCN subtypes. Examples include ZD-7288 (**1**), Zatebradine (**2**), and Ivabradine (**3**) (Fig. 1). ZD-7288 has been shown to inhibit injury-induced ectopic neuronal discharges in isolated spinal nerves and in damaged nerves in situ.[1,3,4] ZD-7288 also exhibits anti-allodynic effects in the rat spinal nerve ligation model (Chung model) and in a model of partial sciatic nerve ligation (Seltzer model).[1,3,5] More recently, ZD-7288 has been shown to inhibit spontaneous pain and tactile allodynia resulting from acute insults such as mild thermal burn and skin incision.[5,6] Ivabradine was approved by the European Medicines Agency in 2005 for the treatment of stable angina pectoris and has been shown to be of equal efficacy to the beta-blocker atenolol and calcium channel antagonist amlodipine in treating this disease.[7] While ZD-7288 and ivabradine are potent blockers of HCN1, a lack of selectivity over other HCN channels has prevented determination of the absolute effectiveness of an HCN1 blocker in treating neuropathic pain. The activity of these compounds at HCN4 is problematic for a non-cardiac indication, because blockade of this channel results in bradycardia due to its critical role in sinoatrial node action potential.[8,9] An important advance in the search for subtype selectivity was recently demonstrated in a series of phenylalkylamines.[10] One of the compounds (**4**, Fig. 1) in this study showed HCN1 selectivity over HCN4 (>150-fold) and HCN2 (~30-fold).

In an effort to identify a novel series of HCN isoform selective blockers, we used high-throughput functional assays[13] to screen a proprietary library of compounds and identified an indane with a modest degree of HCN1/HCN4 selectivity (**5**, Fig. 1, pIC_{50} HCN1 = 5.9, pIC_{50} HCN4 = 4.7). Medicinal chemistry efforts on this compound by our laboratory led to the identification of several potent HCN1 blockers having at least 10-fold selectivity for HCN1

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doi:10.1016/j.bmcl.2011.07.051

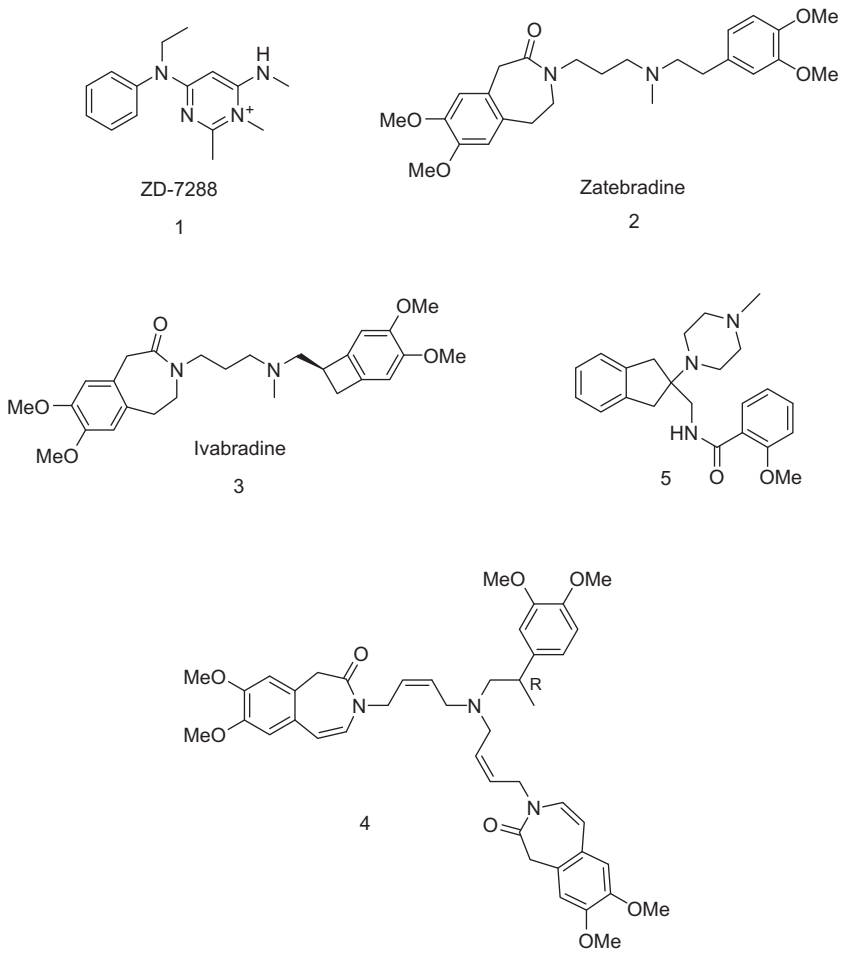


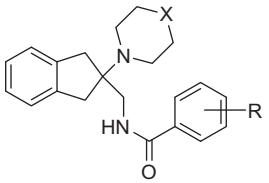
Figure 1. Structures of nonselective HCN1 blockers ZD-7288 (**1**), Zatebradine (**2**), Ivabradine (**3**) and an HCN1 selective indane (**4**).

over HCN4. The routes used to prepare these analogs and their associated biological data are reported below.

Preparation of the indane analogs shown in Table 1 began with a Strecker reaction between indanone, the desired amine and TMSCN to give the nitrile intermediates (**7**) in good yield (Scheme 1).[11] Reduction of the nitrile to the primary amine was problematic, and depending on the nature of the diamine, led to differing amounts of the retro-Strecker by-product (**9**) and desired primary amine (**8**). In many cases, use of LAH as the reducing agent led to significant amounts of the retro-Strecker by-product (**9**). Formation of this unwanted by-product could be minimized in some of those cases by using a mixture of LAH/H_2SO_4 as the reductant.[12] Following isolation, the primary amine was acylated with the desired benzoyl chloride (**10**) to afford the target amides (**11**).

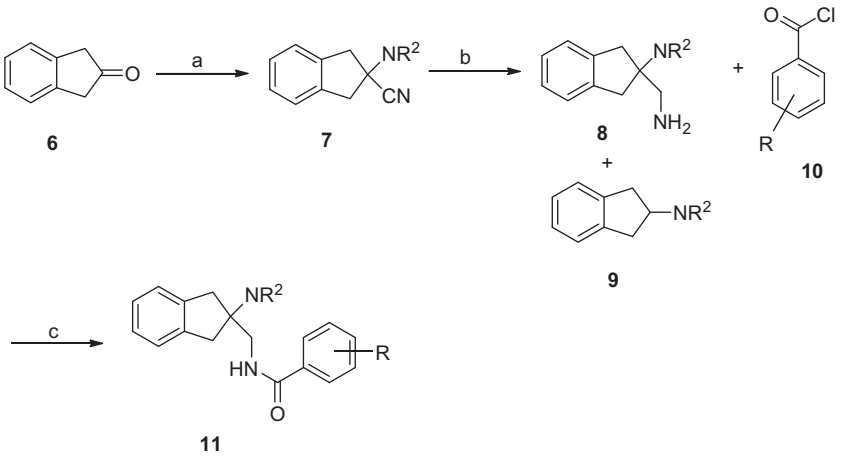
Biological activity of the analogs shown in Tables 1–3 was measured using a high-throughput, functional, VIPR assay.[13] While only moderate HCN1 potency was obtained with this series, it became evident that a substituted piperazine in combination with an alkoxy substituent in the 2-position of the aromatic ring (examples **5, 11a, 11b**, and **11i**) were required for HCN1 potency. Moving the 2-alkoxy group to either the 3 or 4-position (examples **11d** and **11e**), or dealkylation to give a free hydroyl group (example **11g**), resulted in a significant loss in HCN1 potency. Incorporation of an electron withdrawing group, such as the trifluoromethoxy, (example **11c**) also led to a reduction in HCN1 potency. These results led us to postulate that the oxygen of the alkoxy substituent could be making a direct contribution to binding acting as a hydrogen bond acceptor. In addition, replacement of the 2-alkoxy group with electron withdrawing groups, such as chlorine or fluorine (examples **11f** and **11h**), yielded only weak HCN1 blockers. While the data set is limited, it also appears that the presence of a basic nitrogen is critical for HCN1 potency as the two piperidine deriva-

Table 1
pIC_{50} data for indanes at hHCN1 and hHCN4



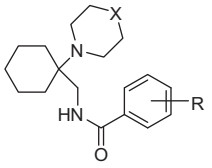
Compds	X	R	hHCN1 (pIC_{50})	hHCN4 (pIC_{50})
ZD-7288	—	—	6.8	7.5
5	*N*-Me	2-OMe	5.9	4.7
11a	*N*-Me	2-OEt	6.4	5.3
11b	*N*-Me	2-OBn	6.2	5.6
11c	*N*-Me	2-OCF_3	4.6	<3.7
11d	*N*-Me	3-OMe	4.2	<3.7
11e	*N*-Me	4-OMe	4.0	<3.7
11f	*N*-Me	2-Cl	4.7	3.8
11g	*N*-Me	2-OH	<3.7	<3.7
11h	*N*-Me	2-F	4.6	<3.7
11i	*N*-*i*Pr	2-OEt	6.2	5.5
11j	CH_2	2-OMe	4.3	<3.7
11k	CH_2	2-OEt	4.8	4.4

pIC_{50} values are the mean of three experiments.



Scheme 1. Reagents and conditions: (a) TMSCN, ZnI_2, Et_2O, NHR_2, MeOH, 80 °C, 8 h, 50–90%; (b) LAH, Et_2O, THF, 2 h, reflux, 0–70% or LAH, H_2SO_4, Et_2O, 5 h, reflux, 0–75%; (c) Pyridine, 50 °C, 24 h, 15–65%.

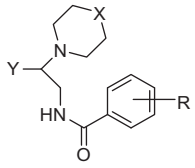
Table 2
pIC_{50} data for cyclohexane core analogs at hHCN1 and hHCN4

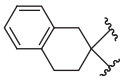
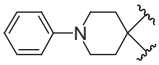
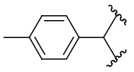


Compds	X	R	hHCN1 (pIC_{50})	hHCN4 (pIC_{50})
12a	*N*-Me	2-OMe	6.4	5.3
12b	*N*-Me	2-OEt	6.9	5.9
12c	*N*-Me	2-Me	4.2	<3.7
12d	*N*-Me	2-Cl	<3.7	4.5
12e	*N*-*i*Pr	2-OEt	7.8	6.5
12f	*N*-*i*Pr	2-OMe	7.5	6.1
12g	*N*-*i*Pr	2-O*i*Pr	6.6	5.8
12h	*N*-*i*Pr	2-Et	<3.7	4.1
12i	*N*-Et	2-OEt	7.5	6.8
12j	*N*-Cyclopropyl	2-OEt	7.3	6.5
12k	*N*-Cyclobutyl	2-OEt	7.1	6.1
12l	*N*-Cyclopentyl	2-OEt	6.7	6.1
12m	NH	2-OEt	6.4	5.4
12n	CH_2	2-OEt	4.8	4.4
12o	O	2-OMe	<3.7	4.3
12p	*N*-Phenyl	2-OEt	<3.7	4.0

pIC_{50} values are the mean of three experiments.

Table 3
pIC_{50} data for cyclohexane core replacements at hHCN1 and hHCN4

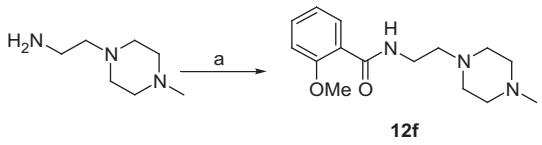


Compds	Y	X	R	hHCN1 (pIC_{50})	hHCN4 (pIC_{50})
13a		*N*-Me	2-OMe	5.8	5.2
13b		*N*-Me	2-OEt	6.4	5.3
13c		*N*-*i*Pr	2-OEt	6.5	5.4
13d		*N*-*i*Pr	2-OMe	6.2	5.3
13e		*N*-Me	2-OMe	5.6	4.7
13f		*N*-Me	2-OMe	<3.7	<3.7

pIC_{50} values are the mean of three experiments.

tives **11j** and **11k**, were devoid of HCN1 activity. Efforts to determine if the proximal piperazine nitrogen was required for HCN1 affinity were not pursued.

Replacement of the indane ring with cylcohexane was accomplished by substitution of indanone with cyclohexanone in Scheme 1 yielding compounds **12a–p** (Table 2). This led to a significant increase in potency at HCN1, and the 10-fold selectivity over HCN4 was maintained in most cases. As with the indane analogs, an alkoxy group in the 2-position of the aryl ring was required for HCN1 potency. When this alkoxy group was replaced with other simple substituents, a significant loss in HCN1 potency was observed (**12c**, **12d**, and **12h**). Small alkyl substituted piperazine rings led, in some cases, to very potent HCN1 blockers with the best combination being an isopropyl piperazine group and a 2-ethoxy phenyl group (example **12e**). Small, cycloalkyl substituted piperazines also yielded potent analogs (examples **12j** and **12k**); however, as the size of the cycloalkyl group increased the resulting HCN1 potency diminished (example **12l**). It is worth noting that the cyclopropyl analog (**12j**) exhibited good potency in the HCN1 VIPR assay despite the piperazine nitrogen being roughly 100-fold less basic than other analogs such as methyl (**12b**) and isopropyl piperazine (**12h**). The importance of the basicity of the distal nitrogen was probed further by preparation of acylated and sulfonylated analogs, which resulted in a significant loss of HCN1 potency (data not shown); however, as mentioned previously, a full investigation of the distal nitrogen for HCN1 blocking potential was not undertaken. A metabolite i.d. study of the *N*-Me piperazine analog **11a**, where the parent compound was incubated with human liver microsomes, showed the primary route of metabolism to be N-demethylation leading to the unsubstituted piperazine metabolite. Based on this result, the analogous cyclohexane analog **12m** was prepared with the hope that it would possess a higher degree of metabolic stability in human liver microsomes relative to the parent methyl piperazine analog **12b**. In fact, **12m** did exhibit an



12f

Scheme 2. Reagents and conditions: (a) 2-Methoxybenzoyl chloride, pyridine, 50 °C, 24 h, 72%.

improved metabolic stability profile as measured by human liver microsomes, having a $t_{1/2}$ of 235 min versus a $t_{1/2}$ of 22 min for **12b**. Unfortunately, this structural modification did lead to a loss in HCN1 potency relative to **12b** and **12e**. In the case of the cyclohexane analogs, replacement of the substituted piperazine group with a simple piperidine ring (example **12n**) led to a complete loss of HCN1 potency, paralleling similar results in the indane series. Not all substitution on the piperazine ring was tolerated. Attachment of a phenyl ring to the terminal nitrogen led to a complete loss of HCN1 potency (example **12p**), further supporting the theory that the presence of a basic nitrogen in this region is important, and contributes to the potency of the series.

Other cores besides indane and cyclohexane were also investigated (Table 3). With the exception of compounds **13e** (compound obtained from original screening deck) and **13f**, all analogs were prepared using the route outlined in Scheme 1. Compound **13f** was prepared by acylation of the commercially available *N*-methyl-*N*′-aminoethyl piperazine (Scheme 2).

As can be seen from the data in Table 3, all other cores investigated offered no advantage over the cyclohexane core. The 6, 6-bicyclic system (example **13a**) displayed similar potency to the indane core, while the cyclopentane core (example **13b**) resulted in about a half log loss in activity relative to its cyclohexane counterpart (example **12b**, Table 2). The tetrahydropyran derivative (example **13c**) and phenyl piperidine analog (example **13d**) maintained some HCN1 potency, but were both significantly less active than their corresponding cyclohexane analogs (example **12d** and **12e**, Table 2). Removing some of the conformational constraint in the molecule by converting the quaternary carbon atom to a tertiary carbon (Example **13e**) had a negative impact on HCN1/HCN4 potency. Decreasing the conformational restriction between the benzamide and piperazine groups further by converting the quaternary carbon to a secondary carbon (**13f**), led to a complete loss in HCN1 potency, highlighting the importance of the spatial relationship of these two groups.

Based upon these results, compound **12m** was chosen for further characterization. Inhibition of heterologous human HCN2 and HCN3 were measured in the functional assay[13] (pIC_{50} = 5.3 and 5.5, respectively). Experiments were performed in vivo to test the effectiveness of this novel series of HCN1 blockers to alleviate nerve injury-induced tactile allodynia in a spared nerve injury (SNI) model[14] and to evaluate their effects on heart rate, using ZD-7288 for comparison. Figure 2a demonstrates the analgesic efficacy of ZD-7288 (ED_{50} = 3 mg/kg) and the complete lack of therapeutic window with respect to heart rate reduction; significant bradycardia is evident below the analgesic threshold (ED_{20} = 1.3 mg/kg). In contrast, the advantage of compound **12m** (Table 2), is clearly seen in Fig. 2b, where analgesic effects (ED_{50} = 6 mg/kg) are substantially separated from bradycardic effects (ED_{20} = 25 mg/kg). Thus, the selective HCN1 blocker, **12m** provides a therapeutic window for the treatment of neuropathic pain, whereas the nonselective blocker, ZD-7288, cannot be administered in a concentration that would alleviate neuropathic pain without affecting heart rate.

In summary, we disclose a series of novel and selective HCN1 blockers. The 10-fold or higher in vitro selectivity for HCN1 versus

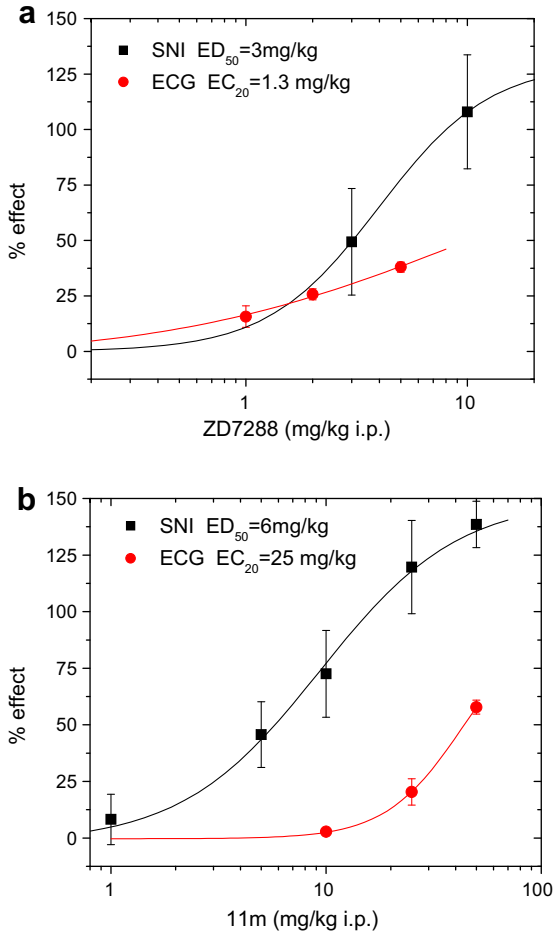


Figure 2. Comparison of the effects ZD-7288 (a) and **12m** (b) on tactile allodynia and bradycardia in mouse.

HCN4 displayed by some of these compounds in a VIPR assay was substantiated by a similar or slightly greater separation in analgesic versus bradycardic potencies. Furthermore, the selectivity profile of these compounds has allowed for testing in a SNI model that clearly showed a contribution of the HCN1 channel to nerve induced allodynia. Compound **12m** represents a novel tool compound for further exploration of the role of HCN subtypes in vivo.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.051.

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