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Modular, efficient synthesis of asymmetrically substituted piperazine scaffolds as potent calcium channel blockers

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ABSTRACT

A novel approach to the synthesis of substituted piperazines and their investigation as N-type calcium channel blockers is presented. A common scaffold exhibiting high activity as N-type blockers is N-substituted piperazine. Using recently developed titanium and zirconium catalysts, we describe the efficient and modular synthesis of 2,5-asymmetrically disubstituted piperazines from simple amines and alkynes. The method requires only three isolation/purification protocols and no protection/deprotection steps for the diastereoselective synthesis of 2,5-dialkylated piperazines in moderate to high yield. Screening of the synthesized piperazines for N-type channel blocking activity and selectivity shows the highest activity for a compound with a benzhydryl group on the nitrogen (position 1) and an unprotected alcohol-functionalized side chain.

Voltage-gated calcium (Ca^{2+}) channels play important physiological roles ranging from the control of cellular excitability and muscle contraction to the release of neurotransmitters and hormones. Based upon biophysical and pharmacological criteria, there are four distinct subtypes of high voltage-activated Ca^{2+} channels (L-, N-, P/Q- and R-types) and a further class of low voltage-activated channels (called T-type).[1] Of note concerning pain processing, in the primary afferent nociceptive pathway N-type Ca^{2+} channels are located at a subset of terminals in the dorsal horn of the spinal cord where they play a crucial role in the release of pro-nociceptive neurotransmitters and neuropeptides.[2] As such, the N-type Ca^{2+} channel is particularly interesting as a clinical target and over the past two decades there has been considerable interest in generating selective N-type channel blockers aimed at the inhibition of spinal neurotransmitter release and the attenuation of afferent pain signals.[3] ω-Conotoxin MVIIA and its synthetic analog Ziconotide (Prialt®) were the first described selective N-type Ca^{2+} channel blockers for pain intervention, although their use clinically is restricted to intrathecal administration due to their polypeptidic structure.[4] There has been a subsequent concerted effort to design and synthesize small molecules that are selective for the N-type Ca^{2+} channel in order to provide both state-dependent blockade and oral availability.[5]

Some of the most common scaffolds that have shown promise for developing N-type channel blockers are N,N′-disubstituted piperazines (Fig. 1).[6] Piperazine itself as well as 2,5-dimethylpiperazine are symmetric, commercially available, inexpensive compounds, which makes their synthetic elaboration for medicinal chemistry attractive. Furthermore, by varying chemical and physical properties aimed at the N-type channel target, preferred compounds have substituents on both nitrogens, which historically has resulted in highly lipophilic compounds.[7] More recent achievements have illustrated the favorable impact of asymmetrically substituted 2-methylpiperazines.[6c] For example, Figure 2 shows a compound that is active for N-type channel blockade and notably more selective over the L-type Ca^{2+} channel and hERG potassium channel than its unmethylated analog.[6c] These results illustrate that without investigating the effect of varied substituents on the piperazine scaffold, significant limitations remain in exploring molecular and functional diversity within this class of promising compounds.

2,5-Unsymmetrically disubstituted piperazines can be accessed via reduction of related diketopiperazines, and several of such amino acid derived products are presently commercially available.[8] While well-established, their syntheses are typically achieved using commercially available amino acids and their derivatives (e.g., 2-aminoalcohols[9] and 1,2-diamines[10]). Most of these synthetic approaches result in substantial waste generation due to requisite sequential protection/deprotection steps[11] and/or the

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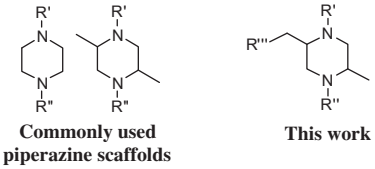


Figure 1. Comparison of piperazine scaffolds.

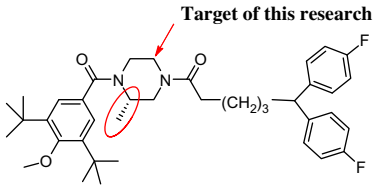
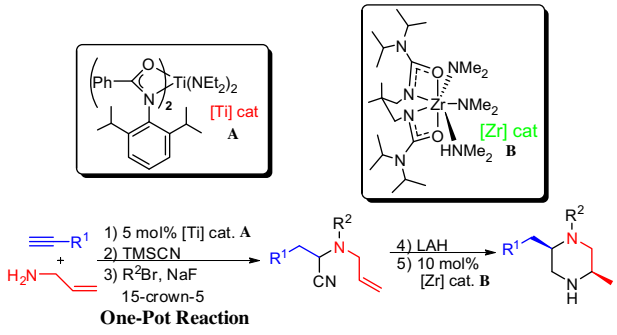


Figure 2. Lead compound of a previous generation of piperazines.[6c]

use of stoichiometric amino acid coupling reagents.[12] Alternatively, the Ugi reaction, an efficient multi-component coupling reaction, can be used to rapidly assemble the precursor diketopiperazines. Unfortunately, the products of this reaction are mixtures of diastereomers that require further purification.[13] Here we show that recently developed group 4 metal hydroamination catalysts can be used for the atom-economic and diastereoselective synthesis of 2-alkyl-5-methyl-substituted piperazines from simple, commercially available alkyne and amine starting materials. By combining a one-pot reaction to access the requisite α-aminonitrile intermediates, followed by reduction and then catalytic ring closure via hydroamination, such unsymmetrically substituted piperazines can be prepared with only 3 isolation/purification protocols and no protection/deprotection steps.[14] Most importantly, the 2-alkyl substituent can be derived from a broad range of terminal alkynes ensuring that this protocol is not limited to amino acids. This modular synthetic approach from readily available starting materials has allowed for the investigation of previously unexplored piperazine scaffolds as N-type channel blockers.

We have been interested in the development of efficient early transition metal hydroamination catalysts for applications in organic synthesis. The combination of low toxicity and low cost of these reactive metals make them attractive for use in the preparation of pharmaceuticals. Our previous work resulted in the development of a very efficient titanium bis(amidate) precatalyst A (Scheme 1), exclusively providing aldimines from a variety of commercially available terminal alkynes and amines.[15]

Other recent investigations have led to the development of a zirconium precatalyst B with a tethered bis(ureate) ligand (Scheme 1).[16] Intramolecular diastereoselective Markovnikov hydroamination of aminoalkenes with this catalyst provides an



Scheme 1. General scheme for efficient and modular synthesis of 2,5-asymmetrically substituted piperazines from simple alkynes and amines.

efficient route into a variety of heterocycles.[16] Most importantly, this catalyst is tolerant to a number of functional groups that may be incorporated into aminoalkene substrates.[17]

By combining these two hydroamination technologies into an efficient approach for the synthesis of 2,5-substituted piperazines, we recently disclosed the facile preparation of a number of heterocycles.[17] Scheme 1 illustrates our synthetic approach. The first three steps are conducted in a one-pot fashion to complete: (a) regioselective hydroamination in the presence of a titanium catalyst,[15a] (b) the addition of trimethylsilyl cyanide to the imine[18] and (c) substitution of the TMS group with a benzyl or benzhydryl derivative. After the purification of the α-aminonitriles the second reaction is the reduction of the cyano group to the primary amine.[19] Finally, to complete the piperazine core, intramolecular and diastereoselective hydroamination of the N-containing aminoalkene is performed using zirconium catalyst B.[16,17]

This efficient and modular approach toward the synthesis of *cis*-unsymmetrically substituted pure piperazines provides the opportunity to prepare classes of previously undescribed compounds for biological screening. Moreover, piperazines furnished from this route contain an unsubstituted nitrogen, which may potentially reduce lipophilicity to result in Ca^{2+} channel blockers with more favourable physiochemical characteristics. Alternatively, the unsubstituted nitrogen may be a site for further selective functionalization.

N-type and L-type Ca^{2+} channel blocking affinities were determined using HEK cells stably co-expressing either the rat brain $Ca_V2.2$ N-type complex or the rat cardiac $Ca_V1.2$ L-type complex together with the $K_{ir}2.3$ potassium channel. A high-throughput FLIPR assay was performed using the fluorescent Ca^{2+} indicator dye, Fluo-4, and with each compound examined by an 8 point concentration-dependent response profile (0.003–10 μM). Membrane potential and channel state were controlled via altering the external potassium ion concentration.[14,20,21]

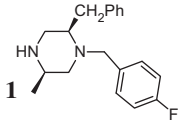
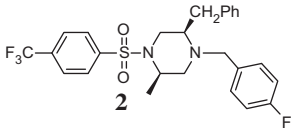
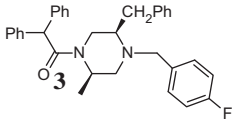
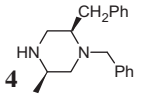
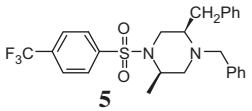
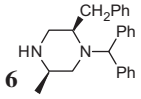
Initial investigations focused on the use of phenylacetylene as a starting material, which resulted in a benzyl group in the 2-position of the piperazine core (Scheme 1, R^1 = Ph). These benzylmethylpiperazines were screened against the N-type and L-type Ca^{2+} channels (Table 1). Overall, the N-type blocking affinities and selectivity against the L-type channel of both N,N′-disubstituted and N-monosubstituted piperazines were generally unfavorable, with the best compound being the benzhydryl-N-monosubstituted piperazine (compound **6**).

Due to the low activity of the 2-benzylpiperazines we next took advantage of our modular protocol to vary position 2 of the piperazine core, while comparing benzyl and benzhydryl groups as N-substituents (Table 2). These changes could be easily achieved with the method described here, starting from commercially available alkynes to easily introduce variable substituents into position 2 (Scheme 1). The screening focused on the N-monosubstituted piperazines based upon the preferred profile of compound **6** and indeed showed improved N-type blocking activity (e.g., compound **11**) although selectivity against the L-type channel was only modest.

This second step of our screening confirmed that changing the substitution pattern from N,N′-disubstituted to N-monosubstituted and thus significantly varying the overall molecular geometry (Fig. 3) could potentially lead to more active compounds. Furthermore, piperazines with a hydrogen bonding functionality in position 2 (compounds **7** and **11**) are the most active. Once again, benzhydryl substituent (compounds **10** and **11**) as compared to the benzyl group (compounds **7**–**9**) yields piperazines that are more potent inhibitors.

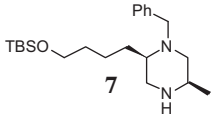
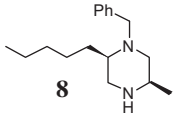
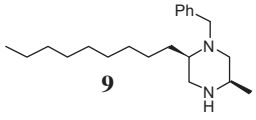
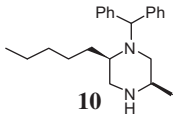
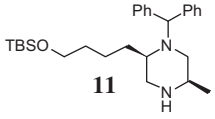
In a final series we examined *N*-benzhydryl piperazines with the hydrogen bonding substituents at varying distances from the piperazine core. This was easily achieved by using starting materi-

Table 1
N-type and L-type calcium channel blocking affinities for compounds **1**–**6**

Structure	N-type IC_{50} (μM)	L-type IC_{50} (μM)	L/N ratio	Yield[a] (%)
 **1**	8.37	10.0	1.2	75
 **2**	10.0	10.0	1.0	66
 **3**	10.0	ND	—	64
 **4**	10.0	10.0	1.0	82
 **5**	10.0	10.0	1.0	76
 **6**	4.34	1.78	0.4	32

ND = not determined.
[a] Overall isolated yield using alkyne as the limiting reagent.

Table 2
N-type and L-type calcium channel blocking affinities for compounds **7**–**11**

Structure	N-type IC_{50} (μM)	L-type IC_{50} (μM)	L/N ratio	Yield[a] (%)
 **7**	4.85	3.34	0.7	21
 **8**	9.84	9.23	0.9	44
 **9**	10.0	5.6	0.6	24
 **10**	2.40	1.78	0.7	21
 **11**	1.07	1.53	1.4	23

[a] Overall isolated yield using alkyne as the limiting reagent.

als of varying chain lengths in the protected alcohol alkyne starting material. To verify that piperazines with a free N–H functionality are preferred even when another hydrogen bonding site is incorporated in the compound, various N′-substituents were reevaluated.

As observed in Table 3, shortening the protected alcohol chain as well as introduction of the substituent on the second nitrogen, did not enhance the activity of any of the screened piperazine derivatives over compound **11**. However, it is noteworthy that in

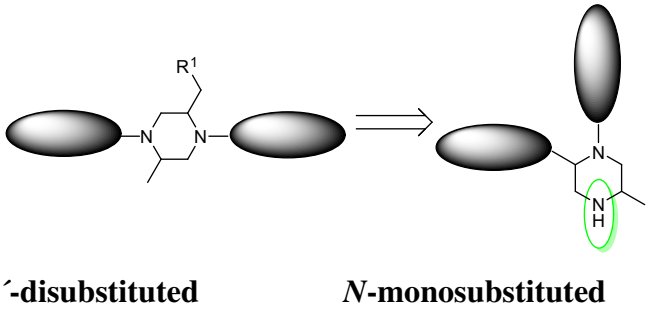
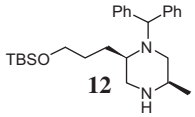
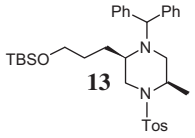
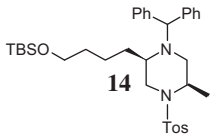
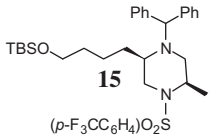


Figure 3. New direction in the development of substituted piperazines.

Table 3
N-type and L-type calcium channel blocking affinities for compounds **12–15**

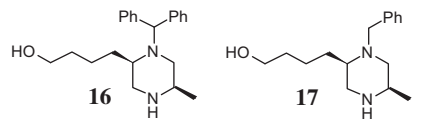
Structure	N-type IC_{50} (μM)	L-type IC_{50} (μM)	L/N ratio	Yield[a] (%)
 **12**	10.0	6.8	0.7	23
 **13**	2.6	5.1	2.0	18
 **14**	4.7	8.2	1.7	20
 **15**	10.0	10.0	1.0	19

[a] Overall isolated yield using alkyne as the limiting reagent.

the case of a shorter protected alcohol chain (compounds **12** and **13**) the *N*-substituent makes the piperazine a more potent N-type channel blocking compound. Furthermore, it was noted that changing the N′-substituent from a tosyl group to a fluorinated derivative resulted in a reduction in N-type channel blocking activity (compounds **14** and **15**).

Under the assay conditions employed, alcohol deprotection may be a concern. Thus, in order to probe the difference between protected and deprotected alcohols, compounds **16** and **17** were prepared and tested (Fig. 4). Deprotected alcohols are less lipophilic and potentially may provide improved physiochemical properties compared to previously synthesized substituted piperazines.[6]

Indeed, one of the TBS-deprotected piperazines (**16**, Fig. 4) showed sub-micromolar (IC_{50} = 0.85 μM) N-type channel blocking affinity, an approximate six-fold selectivity over the L-type channel and a 12-fold state-dependent preference for the N-type channel inactivated state over the N-type closed state (not shown).



N-type IC_{50} = 0.85 μM
L-type IC_{50} = 5.40 μM

N-type IC_{50} = 9.60 μM
L-type IC_{50} = 10.0 μM

Figure 4. Deprotected alcohol substituted piperazines.

In summary, a novel catalytic approach to the synthesis of asymmetrically substituted piperazines has shown significant synthetic potential. The method exhibits good substrate scope, high yields and excellent diastereoselectivity allowing for the generation of a focused library of potential Ca^{2+} channel blockers. The functional screening of a series of novel asymmetrically substituted piperazines revealed (1-benzhydryl-5-methylpiperazin-2-yl)butan-1-ol (compound **16**) to exhibit sub-micromolar N-type channel blocking affinity, state-dependence and promising selectivity over the L-type Ca^{2+} channel. This contribution highlights a modular synthetic approach for the efficient preparation of previously undescribed piperazines suitable for biological screening.

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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.2013.03.114.

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