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Expedited SAR study of high-affinity ligands to the $\alpha_2\delta$ subunit of voltage-gated calcium channels: Generation of a focused library using a solution-phase Sn2Ar coupling methodology

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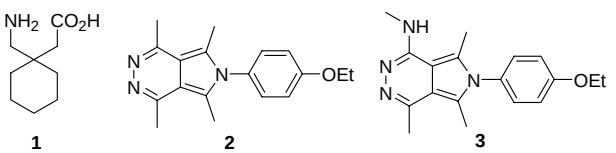
Abstract—The SAR of the lead compound **3**, a novel ligand for the $\alpha_2\delta$ subunit of voltage-gated calcium channels, was rapidly explored. Utilizing a parallel solution-phase Sn2Ar coupling approach, a focused library was obtained. The library was evaluated in vitro and afforded a series of analogues with improved potencies. The SAR trends of the library are also described.

Gabapentin (**1,** neurontin) is an anticovulsant agent employed in clinical treatment of epilepsy.[1] Recent clinical studies have demonstrated that gabapentin is also efficacious in reducing neuropathic pain in humans and suggested that gabapentin and related gamma amino acids may represent promising new therapeutics for treatment of both neuropathic pain[2] and anxiety.[3] Although originally designed as a γ-aminobutyric acid (GABA) analogue, gabapentin was found to have no activity against GABA receptors.[4] Later studies have in fact discovered that gabapentin binds with high affinity to the $\alpha_2\delta$ subunit of voltage-gated calcium channels (VGCCs).[5] It has been postulated that the efficacy of gabapentin in reducing neuropathic pain may be a consequence of its interaction with the $\alpha_2\delta$ subunit.[6]



1 **2** **3**

In an effort to further probe the $\alpha_2\delta$ binding hypothesis and discover a compound superior to gabapentin, we set out to search for non-amino acid small molecules with superior binding affinity to the $\alpha_2\delta$ subunit. High-throughput screening, traditional medicinal chemistry, and high-throughput organic synthesis (HTOS) approaches were employed in this endeavor. The use of solid- and solution-phase chemistry for generation of non-peptidic small molecule libraries has become common practice, in both industry and academia.[7] The approach taken in our laboratories for synthesis of such libraries has been to utilize high-throughput parallel synthesis, either in solution or solid phase, in tandem with rapid purification techniques to afford a series of analogues with improved potency.[8]

In the quest for small molecule gabapentin mimetics, pyrrolopyridazine, **2**, was identified through high-throughput binding assay. **2** displayed an IC_{50} of 180 nM against human A710 cells.[9] Using a traditional medicinal-chemistry approach, a more promising lead 1-methylaminopyrrolopyridazine (**3**) was identified, which possesses an IC_{50} of 40 nM in the $\alpha_2\delta$ binding assay. A medicinal chemistry effort was initiated to delineate the SAR of the *N*-methyl moiety in **3**.[10] However, this initial attempt was limited. Thus to

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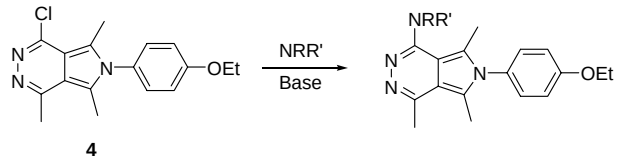


4

Scheme 1.

expedite the SAR, a parallel synthesis approach was employed to rapidly expand the SAR around this moiety. We developed a solution-phase Sn2Ar coupling methodology which allowed us to quickly generate a 1-aminopyrrolopyridazine library to map out the SAR. In this letter, we report the synthesis and in vitro evaluation of this library.

The synthesis of scaffold 1-chloropyrrolopyridazine (**4**) has been described in a previous communication.[10] The last step in the reaction sequence to produce **3** was a Sn2Ar reaction, which is amenable to high-throughput parallel synthesis (Scheme 1).

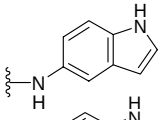
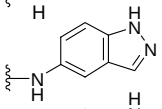
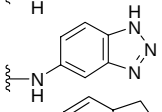
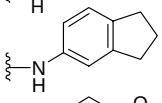
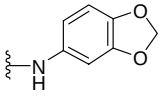
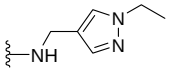
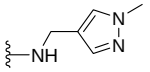
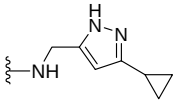
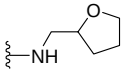
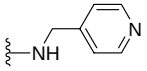
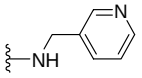
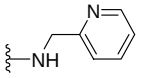
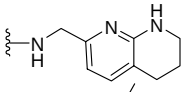
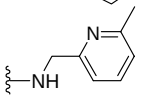
The library synthesis was preformed on an Argonaut Trident Synthesizer. Each reaction vessel in Trident cassettes was pre-loaded with **4** (50 mg) and *N*-methylmorpholine resin (1.1 equiv). Solutions of amines (2 equiv) in pyridine (1 mL) were added to the appropriate reaction vessels using the Trident liquid handler. The resulting suspensions were then shaken on a deck at 100 °C overnight. The reaction mixtures were allowed to cool to room temperature. Additional *N*-methylmorpholine resin (1.1 equiv), chloroformate resin (1.3 equiv), and chloroform (3 mL) were added to scavenge the excess amines. The reactions were capped and shaken on a deck at 60 °C for 4 h. The products were then collected using the Trident liquid handler.

Of the 576 parallel reactions carried out, without further purification, 374 (65%) gave products with purities equal to or greater than 90% by the LC–MS analysis. Yields ranged from 27% to 98%. In addition to LC–MS, a series of randomly selected products were subjected to ^{1}H NMR study to confirm the structures and corroborate the purities. The 374 compounds were used in the in vitro study.

The library was screened in a [^{3}H]gabapentin binding assay against human A710 cell membranes. As a demonstration of the reliability of library screening, compounds **5**, **6**, **18**, **28**, and **32** were re-synthesized, purified, and re-assayed. The deviations of the re-assay from the library screening were <30%.

Summary of the in vitro $\alpha_2\delta$ binding screening results of the library is shown in Tables 1–3. Only compounds with activities less than or equal to 100 nM and compounds with activities greater than 100 nM clearly indicated that a SAR trend has been included. The library targeted several hits with improved or equal potencies compared to those of the lead compound **3** (**5**, **6**, **17**, **19**, and **21**). The culminant series could be divided into distinct trends as reflected in Tables 1–3.

Table 1. In vitro $\alpha_2\delta$ binding affinities for compounds **5**–**18**

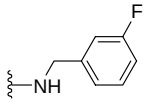
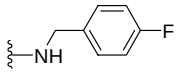
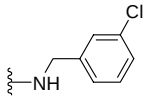
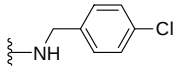
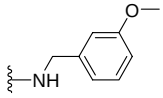
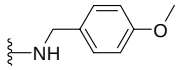
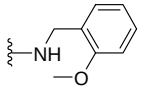
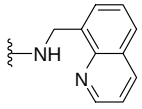
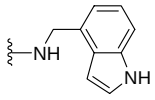
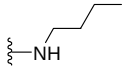
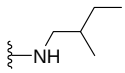
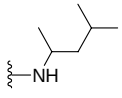
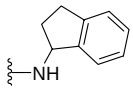
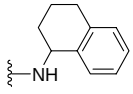
Compound	R	$\alpha_2\delta$ IC_{50} (nM)[a]
5		30
6		36
7		346
8		199
9		207
10		64
11		69
12		68
13		50
14		84
15		119
16		>3000
17		32
18		84

[a] Values are means of three experiments.

In general, Table 1 reflects compounds with heterocyclic substitution patterns.

Indolic (**5**) and indazolic (**6**) substituents are well tolerated in $\alpha_2\delta$ binding, while benzotrazole (**7**) and other five-membered rings on the same position (**8**, **9**) drastically decreased binding affinities. Pyrazole-4-methyl (**10**, **11**) and pyrazole-3-methyl (**12**) are permissive disregarding a different small substituent at N_2 or C_5. Replacing the pyrazole with tetrahydrofuran (**13**) retains the activity. Pyridinylmethyl is yet another intriguing series of heterocyclic substitutions (**14**–**16**). 4- and 3-Pyridinylmethyl are tolerated, while 2-pyridinylmethyl, on the other hand, abolished $\alpha_2\delta$ binding. However, installing

Table 2. In vitro $\alpha_2\delta$ binding affinities for compounds **19**–**33**

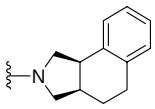
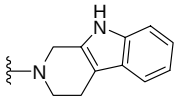
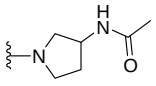
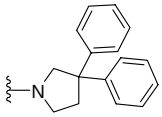
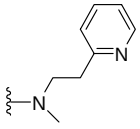
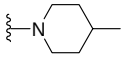
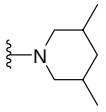
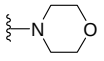
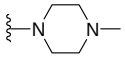
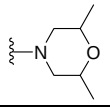
Compound	R	$\alpha_2\delta$ IC_{50} (nM)[a]
19		18
20		94
21		37
22		65
23		239
24		702
25		76
26		63
27		70
28		67
29		61
30		84
31		296
32		54
33		161

[a] Values are means of three experiments.

a fused piperidine ring (**17**) or a methyl at the C_6 position of the 2-pyridinylmethyl (**18**) retained activities.

The second trend reflects compounds that have benzylic substitution patterns (Table 2). The substitution pattern on the benzene ring is crucial. Halogen substituent at *meta* (**19**, **21**) or *para* (**20**, **22**) position is tolerated in the $\alpha_2\delta$ assay with the *meta* position being optimal.

Table 3. In vitro $\alpha_2\delta$ binding affinities for compounds **37**–**48**

Compound	R	$\alpha_2\delta$ IC50 (nM)[a]
34		84
35		135
36		144
37		153
38		179
39		180
40		221
41		370
42		702
43		1692
44		2926
45		>3000

[a] Values are means of three experiments.

While methoxy at the same position (**23**, **24**) diminished the binding activities, it is well tolerated at the *ortho* position (**25**). The union of the benzylic and some heterocyclic functionalities (**26**, **27**) is also permissive.

The third SAR trend addresses small aliphatic substitutions at the amino group. Small aliphatic groups are tolerated (**28**, **29**, and **30**). However, as the aliphatic group becomes bigger (**31**), the $\alpha_2\delta$ bind potency decreased.

The union of small aliphatic group and benzylic group to form constrained aliphatic/benzylic substitution is tolerated. The trend also indicates that a smaller aliphatic ring (**32**) is better tolerated than the larger ring (**33**).

Many secondary amine building blocks were used in the library synthesis to generate tertiary amines. In general,

tertiary amine analogues (Table 3) are less potent than secondary amines (Tables 1 and 2). The top fivemost potent tertiary amines (**34**–**38**) are quite sizable, suggesting the existence of a large pocket adjacent to the pyridazine nitrogen on $\alpha_2\delta$ subunit. The trend of decreasing binding potency, while increasing the size of the aliphatic substituent, is also observed in the tertiary amine series (**39**–**42**). Morpholine and piperizine are detrimental to the binding potency (**43**–**45**).

In summary, through the use of a high-throughput parallel Sn2Ar reaction, we were able to expedite the SAR studies of lead compounds **3**, a gabapentin mimetic. The library targeted a series of leads with improved or equal potencies compared to those of lead compound **3**. More importantly, the substitution of heterocyclic, benzylic, and aliphatic SAR trends and permissive functionalities was defined. Further profiling of the leads **5** and **6** is currently underway.

References and notes

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