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# Synthesis and biological evaluation of 6-aryl-6*H*-pyrrolo[3,4-*d*]-pyridazine derivatives as high-affinity ligands of the $\alpha_2\delta$ subunit of voltage-gated calcium channels

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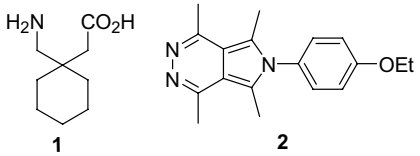
**Abstract**—A novel class of 2*H*-pyrrolo[3,4-*c*]pyridazine ligands of the $\alpha_2\delta$ subunit of voltage-gated calcium channels is described. Compound **4a** with high affinity toward $\alpha_2\delta$ was identified through structure–activity relationship studies of the lead compound. Tritiated ligand [$^3$H]-**4b** was synthesized to demonstrate that this ligand binds to the same site as Gabapentin toward $\alpha_2\delta$ subunit of voltage-gated calcium channels.


## 1. Introduction

Gabapentin (1-(aminomethyl)cyclohexane acetic acid; Neurontin), **1**, is a novel anticonvulsant agent, which is useful as add-on therapy in the treatment of epileptic seizures (Fig. 1).[1] Several clinical reports suggest that Gabapentin has therapeutically beneficial effects against chronic pain states and anxiety.[2] Originally, Gabapentin was designed as a lipophilic $\gamma$-amino butyric acid (GABA) analogue; however, it has since been shown not to interact directly with any of the enzymes in the metabolic pathways of glutamate and GABA at any significant concentration, nor does it bind to any of the GABA receptors.[3] Recently, it has been shown that Gabapentin binds with high affinity to a novel site on the $\alpha_2\delta$ subunit of a calcium channel;[4] it has been proposed that this site may be involved in the mediation of the pharmacological actions of Gabapentin.[5]

Herein, we report the synthesis and evaluation of nonamino acid $\alpha_2\delta$ ligands identified during our screening campaign. The initial lead, pyrrolopyridazine (**2**), identified through a high-throughput [$^3$H]-Gabapentin binding assay, exhibited an $IC_{50}$ of 180 nM in A710 cells.[6] In a previous communication,[7] we documented the SAR result primarily on the pyrrolopyridazine portion of the lead structure **2**. In this paper, we report our continued SAR studies of **2**, with emphasis on the phenyl ring.



1 2

**Figure 1.** Structure of Gabapentin **1** and lead compound **2**.

*Keywords*: Gabapentin; Affinity; SAR.
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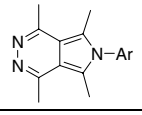
## 2. Results and discussion

Structure–activity studies were focused around modifying the phenyl ring system and directed toward improving the potency of the series. Our initial attempt was to replace the right phenyl ring with various heterocycles such as pyridine, pyridazine, or pyrimidine, afforded analogues with diminished binding affinities to the $\alpha_2\delta$ subunit relative to **2** (Table 1).

As the heterocyclic replacements did not improve potency, we focused on introducing one or more methyl, alkoxy, or halogen substitutions around the phenyl ring as shown in Table 2. Incorporating substituents on the phenyl ring revealed dramatic stereo-electronic effects in the binding affinity of the pyrrolopyridazine lead to $\alpha_2\delta$ subunit. A highly potent compound **4d** ($\alpha_2\delta$ $IC_{50}$ 27 nM) with an *ortho*-methyl substituent was identified. In contrast, the corresponding *meta*-methyl substituted analogue (**4e**, $\alpha_2\delta$ $IC_{50}$ 298 nM) was 10-fold less potent as compared to compound **4a** (Table 2). The presence of the *ortho*-methyl moiety most likely changes the conformation of the molecule by placing the two rings perpendicular to each other such that the phenyl group rotates with respect to the pyrrolopyridazine ring thus providing a comparable nonplanar conformation, resulting in improved binding affinity. However, incorporating additional methyl group, leading to dimethyl analogues, either in the 3-, 4-, or 5-position (**4g**,**h** and **4j**) failed to significantly improve potency.

To further optimize binding affinity of **4e**, SAR was focused around various *ortho*-substituents on the phenyl ring while retaining ethoxy group at the *para*-position. Electron donating groups such as methoxy (**4a**), ethyl (**4b**), vinyl (**4c**), thiomethyl (**4e**), and ethoxy (**4f**) retained

**Table 1.** $\alpha_2\delta$ Binding affinity of compounds **2** and **3a**–**f**



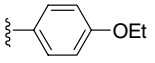
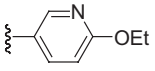
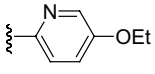
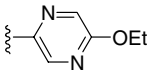
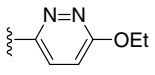
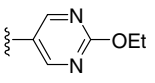
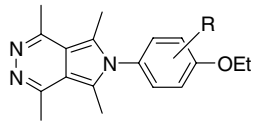
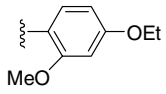
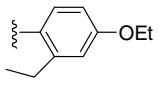
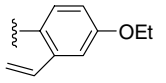
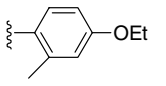
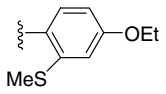
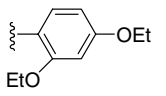
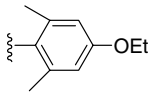
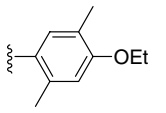
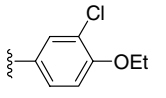
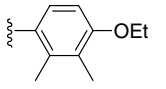
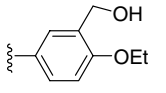
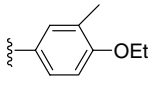
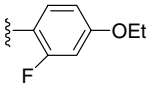
| Compounds | Ar= | $\alpha_2\delta$ Binding $IC_{50}$ (nM) |
|---|---|---|
| **2** |  | 180 |
| **3a** |  | 2100 |
| **3b** |  | 5500 |
| **3c** |  | 6100 |
| **3d** |  | 7600 |
| **3f** |  | >10000 |

**Table 2.** $\alpha_2\delta$ Binding affinity of compounds **2** and **4a**–**m**



| Compounds | R | $\alpha_2\delta$ Binding $IC_{50}$ (nM) |
|---|---|---|
| **4a** |  | 4 |
| **4b** |  | 13 |
| **4c** |  | 25 |
| **4d** |  | 27 |
| **4e** |  | 28 |
| **4f** |  | 33 |
| **4g** |  | 54 |
| **4h** |  | 94 |
| **4i** |  | 100 |
| **4j** |  | 282 |
| **4k** |  | 292 |
| **4l** |  | 298 |
| **4m** |  | 313 |

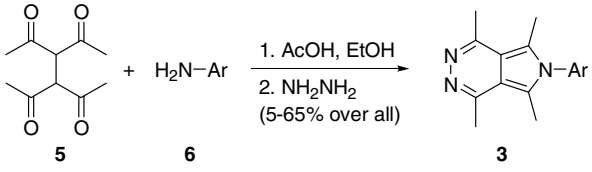
or improved binding affinity significantly; the methoxy group provided the most potent compound (**4a**, $IC_{50}$ 4 nM) in this series. Ligand **4a** showed a 45-fold increase in potency over the initial lead **2**. Various electron withdrawing groups at *ortho*-position, exemplified by **4m**, led to significant decrease in potency. In general, analogues with electron donating groups were preferred, and *ortho*-substituents were preferred relative to *meta*-substituents.

## 3. Chemistry

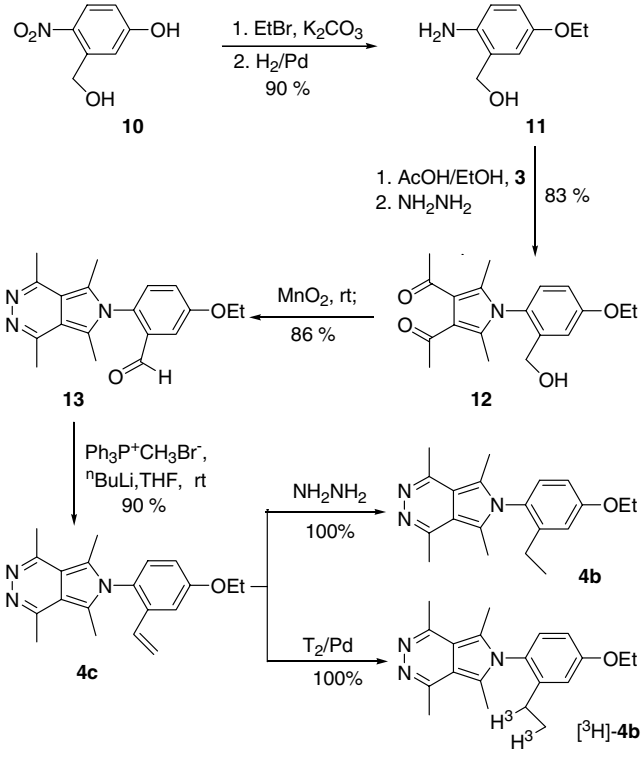
The general synthetic approach for the synthesis of analogues of **3** is shown in Scheme 1. Condensation of a variety of anilines **6** with commercially available tetraketone **5** provided corresponding diacetyl pyrroles.[8] Treatment of diacetylpyrroles with excess of hydrazine in ethanol at room temperature over 30 min, followed by quenching with water afforded the desired pyrrolopyridazines **3** after simple filtration.

The chemical synthesis of analogues **4a** and **4f** is outlined in Scheme 2. Condensation of 2,4-dimethoxy aniline **7** with tetraketone **3** afforded pyrrole **8**. Regioselective removal of *para*-methoxy group of the phenyl ring was achieved by treating compound **8** with boron tribromide (2.0 equiv, 0 °C), to afford **9a** and **9b** in 80% and 15% yield, respectively.[9] Compounds **9a** and **9b** were mono and bis-ethylated under standard procedure, and the resulting intermediates were treated with hydrazine to afford compounds **6a** and **6e** in 90% yield over two steps.

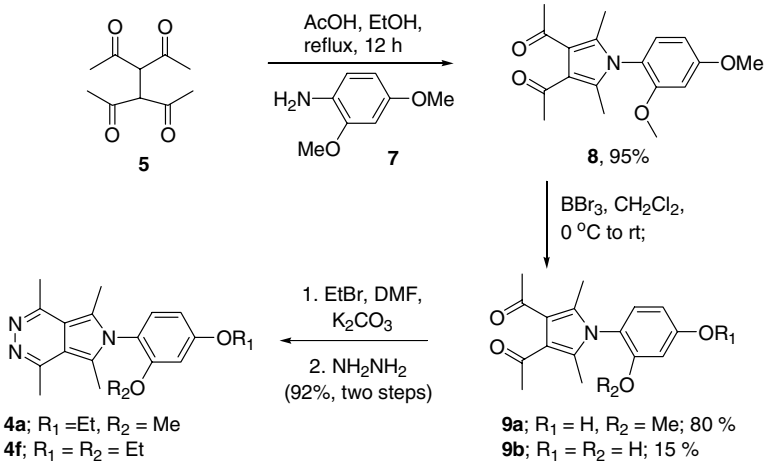
To establish that binding affinity of the compounds is specific for $\alpha_2\delta$ subunit, the radio ligand [$^3$H]-**4b** was prepared from the commercially available (2-amino-5-ethoxyphenyl)-methanol **10** (Scheme 3). Compound **10** was ethylated, followed by reduction ($H_2$/Pd) of the nitro group to afford the free amine **11**. This crude material was then condensed with tetraketone **5**, followed by subsequent treatment with hydrazine to yield




**Scheme 1.**




**Scheme 3.**




**Scheme 2.**

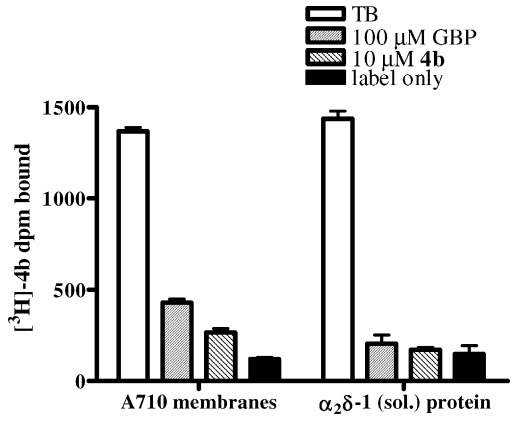



**Figure 2.** Specific binding of [$^3$H]-**4b** to $\alpha_2\delta$ subunit.

compound **12** (83% yield). This allylic alcohol was oxidized with manganese dioxide at room temperature to generate the corresponding aldehyde **13**. The aldehyde was then converted to olefin **4c** under Wittig conditions to yield **4c** in 90% yield. Treatment of **4c** with excess hydrazine (30 equiv, EtOH, rt) for 2 h resulted in complete reduction of the vinylic double bond to afford ligand **4b** (100%).[10] Tritiated ligand [$^3$H]-**4b** was then prepared from **4c** via standard tritiation procedure ($T_2$/Pd).

The ligand [$^3$H]-**4b** was used to evaluate the specific binding affinity of these compounds toward $\alpha_2\delta$ subunit of voltage-gated calcium channels and also in A710 cell membrane (Fig. 2). [$^3$H]-**4b** showed high specific binding ($K_d = 4$ nM) to soluble human $\alpha_2\delta$ and in A710 cell. Ligand [$^3$H]-**4b** was completely displaced by Gabapentin and cold **4b** in soluble version of the protein and in A710 cells membrane, suggesting that these nonamino acid ligands are Gabapentin mimics in this in vitro assay.

In summary, further SAR on the phenyl ring lead to identification of *ortho*-substituents (**4a**,**b**) that improved potency by 10- to 40-fold over the lead compound **2**. Tritiated analogue of **4b** was successfully synthesized and evaluated in the Gabapentin binding assay. This allowed us to demonstrate that these ligands bind with high affinity and are Gabapentin mimetic under the described assay conditions.

## References and notes

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6. [$^3$H]-Gabapentin binding assay in human brain membrane (A710 membrane) was adopted from the method in the literature. A710 cells are defined as HEK 293 cells stably transfected with human α1B, β3a, and $\alpha_2\delta$-1b subunits. Suman-Chauhan, N.; Webdale, L.; Hill, D. R.; Woodruff, G. N. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1993**, *244*, 293.
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8. All intermediates and target compounds provided satisfactory $^1$H and LCMS spectra, and elemental analysis.
9. Interestingly, monodemethylation of the *ortho*-methoxy group of compound **10** was effected using EtSNa/DMF reaction conditions. For reference, see: Lal, K.; Ghosh, S.; Salomon, R. *J. Org. Chem.* **1987**, *52*, 1072–1078.
10. We found that this metal-free double bond reduction condition was general in other reaction systems. Detailed discussions regarding the scope and limitation will be reported in due course.