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

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Abstract—A novel class of 6-aryl-6*H*-pyrrolo[3,4-*d*]pyridazine ligands for the $\alpha_2\delta$ subunit of voltage-gated calcium channels has been described. Substitutions in the aryl ring of the molecule were generally not tolerated, and resulted in diminished binding to the $\alpha_2\delta$ subunit. Modifications to the pyridazine ring revealed numerous permissive substitutions, and detailed SAR studies were carried out in this portion of the molecule. Replacement of the pyridazine ring methyl group with an aminomethyl functionality provided greatly improved potency over the initial lead. The initial lead compound displayed good rat pharmacokinetic properties, and was shown to be efficacious in the Chung model for neuropathic pain in rats.

1. Introduction

Gabapentin (**1**, Neurontin) is a clinically prescribed anticonvulsant agent employed for the treatment of epilepsy.[1] Recent studies have suggested that gabapentin and related GABA analogue pregabalin (**2**) may represent promising new therapeutic agents for treatment of both neuropathic pain[2] and anxiety.[3] Furthermore, efficacy for both Gabapentin and pregabalin (**2**) has been demonstrated in clinical neuropathic pain in humans (Fig. 1).[4]

Although originally designed as a constrained GABA analogue, gabapentin was found to have no activity at GABA receptors.[5] Exposure of ^{3}H-gabapentin to porcine cerebral cortex membranes resulted in the identification of a single high affinity binding site, the $\alpha_2\delta$ subunit of voltage gated calcium channels (VGCCs).[6] Although various hypotheses have been put forth for the in vitro functional consequences of binding to the $\alpha_2\delta$ subunit of VGCCs, neither a signal transduction pathway nor a functional consequence of $\alpha_2\delta$ binding have been unambiguously defined. Despite this fact, it has been postulated that the efficacy of gabapentin in neuropathic pain models may be a result of interactions with the $\alpha_2\delta$ subunit.[7]

A number of publications have disclosed extensive SAR studies around the general gabapentin structure.[8] Our

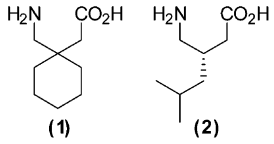

Figure 1. Gabapentin (**1**) and Pregabalin (**2**).

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goal was to probe the $\alpha_2\delta$ hypothesis further by searching for non amino acid small molecules with binding affinity to the $\alpha_2\delta$ subunit in an effort to further understand the pharmacology of gabapentin. Pyrrolopyridazine (**3**) was identified through a high-throughput binding assay in a program directed at the discovery of non amino acid gabapentin mimetics. Utilizing ^{3}H-gabapentin as the radioligand, compound (**3**) displayed an IC_{50} of 180 nM in rat $\alpha_2\delta$ membranes (Fig. 2).

This compound was an attractive lead from a medicinal chemistry point of view, and in this communication, we report the synthesis and biological evaluation of variants of this structure.

2. Chemistry

The general synthetic approach towards the synthesis of analogues of **3** is shown in Scheme 1. Condensation of a variety of substituted anilines (**5**) with commercially available tetraketone (**4**) provided corresponding diacetyl pyrroles.[9] These crude pyrroles were exposed to excess hydrazine in ethanol at room temperature. The resulting pyrrolopyridazines were isolated in good yield by filtration after dilution of the reaction mixtures with water.

Modifications in the pyridazine ring of the molecule were accomplished with the chemistry shown in Scheme 2.

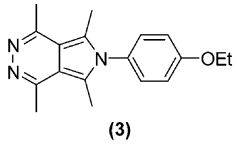
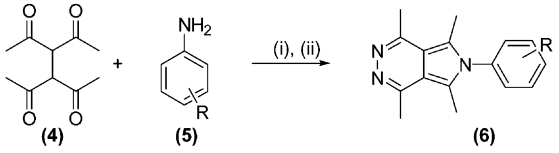
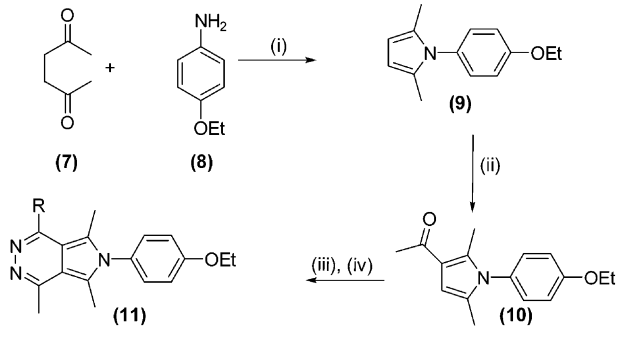


Figure 2. Pyrrolopyridazine (**3**).



Scheme 1. Reagents: (i) AcOH, PhMe, Δ; (ii) H_2NNH_2, EtOH, (60–99%).

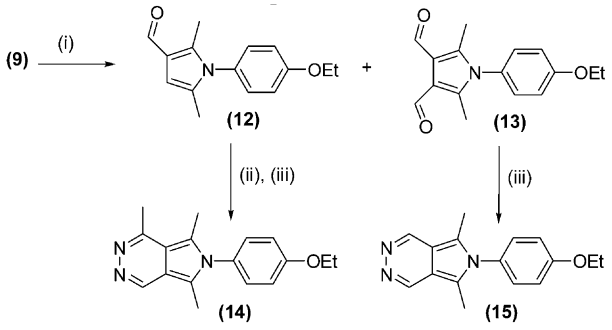


Scheme 2. Reagents: (i) AcOH, PhMe, Δ; (ii) Ac_2O (80%); (iii) RCOCl, $SnCl_4$, CH_2Cl_2; (iv) H_2NNH_2, EtOH (65–90%).

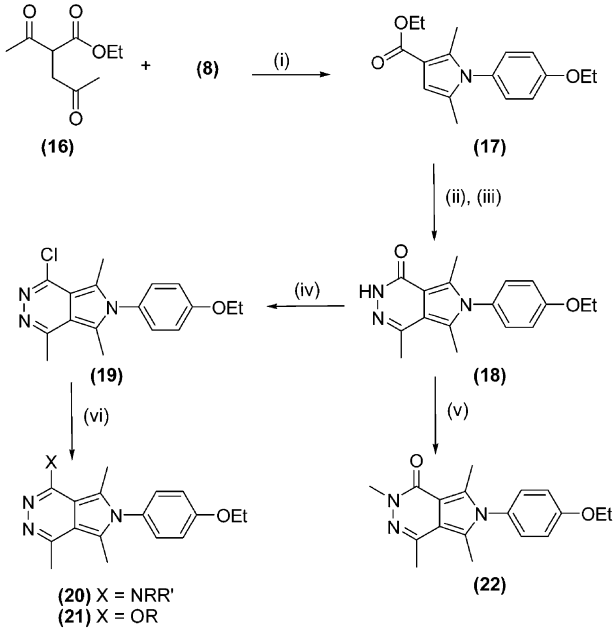
Condensation of 2,5-hexanedione (**7**) with *p*-phenetidine (**8**) afforded dimethylpyrrole **9**. Exposure of **9** to the action of acetic anhydride smoothly provided monoacetyl pyrrole **10**.[10] Acetylpyrrole **10** was reacted with a variety of acid chlorides in the presence of $SnCl_4$. This afforded the intermediate diacyl pyrroles, which were condensed with hyrdazine in EtOH to afford substituted pyrrolopyridazines **11**.

Removal of one or both of the methyl groups adjacent to the pyridazine was accomplished as shown in Scheme 3. Exposure of **9** to the action of the Vilsmeier reagent afforded a 9:1 mixture of mono and diformyl pyrroles **12** and **13**. Intermediate **12** was reacted with acetyl chloride and $SnCl_4$, followed by hydrazine to afford **14**. Intermediate **13** was reacted with hydrazine to afford **15**.

Installation of heteroatoms adjacent to the pyridazine was accomplished utilizing the chemistry outlined in Scheme 4. Condensation of *p*-phenetidine with ethyl 2-acetyl-4-oxovalerate[11] (**16**) afforded ester **17**. Acylation with acetyl chloride and $SnCl_4$, followed by conden-



Scheme 3. Reagents: (i) $POCl_3$, DMF, 90%; (ii) AcCl, $SnCl_4$, CH_2Cl_2, 85%; (iii) H_2NNH_2, EtOH (99%).



Scheme 4. Reagents: (i) AcOH, PhMe, Δ; (ii) AcCl, $SnCl_4$, CH_2Cl_2; (iii) H_2NNH_2, EtOH (75%); (iv) $POCl_3$, Δ, 95%; (v) NaH, MeI, DMF, 49%; (vi) NHRR′ or NaOR, DMF (30–65%).

sation with hydrazine afforded pyridazinone **18**.[12] This intermediate was *N*-methylated by exposure to sodium hydride and methyl iodide to afford **22**. Alternatively, exposure of **18** to $POCl_3$ provided chloropyridazine **19**. The corresponding 2-amino (**20**) or 2-alkoxypyridazines (**21**) were prepared by reacting chloride **19** with either amines or sodium alkoxides in DMF.

3. Biological results

Initial exploration into the primary lead **3** focused on the right-hand ethoxyphenyl ring. Representative SAR information from the right-hand portion of the molecule is shown in Table 1. Compounds were profiled in a binding assay utilizing ^{3}H-gabapentin as the radioligand.[13] In general, replacement of the ethoxyphenyl group of **3** provided analogues with diminished $\alpha_2\delta$ potency relative to **3**. Shifting the ethoxy group from the 4 position to the 3 position (**6a**) or replacement of the ethoxy group with a methoxy group (**6d**) abolished $\alpha_2\delta$ binding. However, substituents in the 2-position appeared to be tolerated, as 2-ethoxy derivative **6b** showed only a 10-fold loss in potency at the $\alpha_2\delta$ protein relative to **3**.

Given the tight SAR revealed in the ethoxyphenyl ring, efforts were shifted to the pyrrolopyridazine portion of the molecule. Replacement of one (**14**) or both (**15**) pyridazine ring methyl groups with hydrogens abolished $\alpha_2\delta$ binding. Installation of groups larger than methyl in this position was tolerated. Table 2 shows representative SAR information on the pyridazine methyl group replacements made in **3**. Replacement of the methyl group in **3** with a phenyl group afforded **11a** ($\alpha_2\delta$ IC_{50} 382 nM), suggesting the existence of a lipophilic pocket adjacent to the pyridazine nitrogen. Scanning substituents on the newly introduced phenyl ring demonstrated significant stereoelectronic effects in the binding affinities of the pyrrolopyridazine ligands to the $\alpha_2\delta$ subunit. In general, analogues with electron donating groups were preferred relative to electron withdrawing groups, and *para* substituents were preferred relative to *meta* and *ortho* substituents. Potency tracked well with this trend: 4-OMe > 3-OMe > 2-OMe4-Cl > 3-Cl > 2-Cl. The optimal substituent identified in the phenyl ring was a 4-methyl group (**11b**) with a binding affinity of 86 nM. Substituents as large as a phenyl group (**11k**) were tolerated ($\alpha_2\delta$ IC_{50} 1200 nM). A variety of alkyl and cycloalkyl groups were installed in this position (**11o–x**) and displayed good binding affinity to $\alpha_2\delta$.

Replacement of the pyridazine methyl group of **3** with heteroatoms provided more promising improvements in binding affinity. Compound **20a** in which the pyridazine methyl group of **3** was replaced with an *N*-methyl group showed a significant increase in potency. The corresponding ring-alkylated isomer **21** was significantly less potent, and the corresponding keto tautomers **22** and **18** did not bind to $\alpha_2\delta$. Dimethylamino derivative **20b** was significantly less potent than the corresponding monomethyl amine **20a** (Table 3).

Groups as large as aryl were tolerated on the nitrogen (**20c**, **20e**, and **20f**). Introduction of polar substituents on the aryl ring such as amino groups (**20b**) or pyridyl groups (**20g**) diminished $\alpha_2\delta$ binding.

Table 1. $\alpha_2\delta$ Binding affinities for compounds **3** and **6a–e**

Compd	R	$\alpha_2\delta$ IC_{50}, nM[a]
3	4-OEt	180
6a	3-OEt	> 10,000
6b	2-OEt	1850
6d	4-OMe	> 10,000
6e	4-*O*-*n*-Pr	5180

[a] Values are means of three experiments.

Table 2. $\alpha_2\delta$ Binding affinities for compounds **11a–x**

Compd	R	$\alpha_2\delta$ IC_{50}, nM[a]
11a	Ph	382
11b	4-Methylphenyl	86
11c	4-Methoxyphenyl	154
11d	3-Methoxyphenyl	335
11e	2-Methoxyphenyl	737
11f	4-Chlorophenyl	701
11g	3-Chlorophenyl	1597
11h	2-Chlorophenyl	4367
11i	2,4-Dimethoxyphenyl	597
11j	4-Ethylphenyl	107
11k	*p*-Biphenyl	1263
11l	4-Ethoxyphenyl	153
11m	4-Trifluoromethoxyphenyl	1729
11n	*p*-Fluorophenyl	528
11o	Et	250
11p	2-Cyclopentylethyl	148
11q	*n*-Pentyl	360
11r	3-Methylbutyl	263
11s	2-Methylpropyl	171
11t	2,4,4-Trimethylpentyl	577
11u	Cyclopentylmethyl	211
1v	Cyclopentyl	185
11w	Cyclohexyl	235
11x	Cyclopropyl	170

[a] Values are means of three experiments.

Table 3. $\alpha_2\delta$ Binding affinities for compounds **20a–g** and **21**

Compd	R	R′	$\alpha_2\delta$ IC_{50}, nM[a]
20a	Me	H	40
20b	Me	Me	170
20c	Ph	H	75
20d	4-NH_2-Ph	H	> 10,000
20e	4-OMe-Ph	H	440
20f	4-Me-Ph	H	240
20g	4-Pyridyl	H	> 10,000
21	Me	n/a	2181

[a] Values are means of three experiments (n/a = not applicable).

Table 4. Selected rat pharmacokinetic parameters for compound **3**

Dose/Route	2 mg/kg iv	10 mg/kg po	20 mg/kg ip
%F	n/a	81%	n/a
AUC (μM*h)	6.4	27	141
C_{max} (μM)	n/a	3.6	101
T_{max} (h)	n/a	2.8	0.3
$T_{1/2}$ (h)	0.91	4.5	1.2
Clp (mL/min/kg)	18.5	n/a	n/a
Vdss (L/kg)	1.3	n/a	n/a

n/a, not applicable.

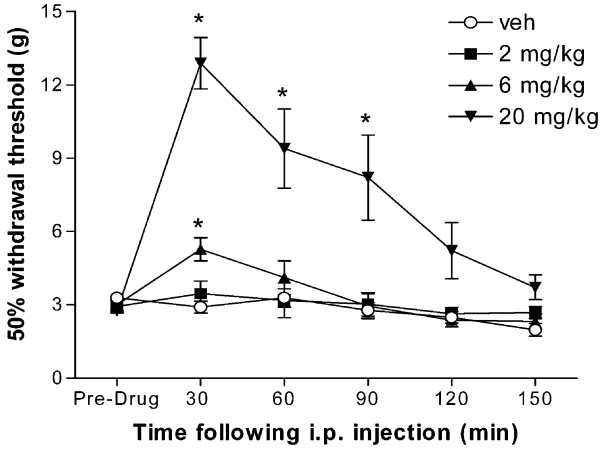


Figure 3. Data for compound **3** in the rat Chung model.

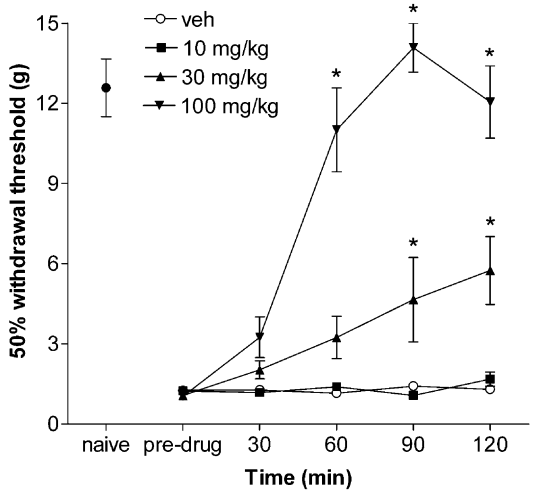


Figure 4. Data for gabapentin (**1**) in the rat Chung model.

Compound **3** was examined in rat pharmacokinetic assays and was found to have excellent pharmacokinetic parameters.[14] Doses of 20 mg/kg ip resulted in plasma concentrations of 100 μM and an AUC of 141 μM*h. The compound displayed excellent oral bioavailability, and low clearance (Table 4).

In light of the favorable pharmacokinetic profile of **3**, we were compelled to examine the performance of the compound in the rat Chung model[15] (neuropathic pain). Figures 3 and 4 shows Chung model data for compound **3** ($n=6$ rats per treatment group). In a dose-dependent fashion, **3** provided full reversal of tactile allodynia in rats. An ID_{50} of 9.4 mg/kg was obtained after intraparitoneal dosing. This compound was slightly more potent than gabapentin (**1**, $ID_{50}=39$ mg/kg ip, $n=6$ per treatment group) (shown in Fig. 3) in the Chung model.

4. Conclusion

In conclusion, a novel series of non-amino acid $\alpha_2\delta$ ligands has been disclosed. Like gabapentin and pregabalin, compound **3** displays efficacy in a preclinical model for neuropathic pain. Further profiling of these novel compounds is currently underway.

References and notes

1. Bryans, J. S.; Wustrow, D. J. *Med. Res. Rev.* **1999**, *19*, 149.
2. Pan, H. L.; Eisenach, J. C.; Chen, S. R. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 1026. Hunter, J. C.; Gogas, K. R.; Hedley, L. R.; Jacobsen, L. O.; Kassotakis, L.; Thompson, J.; Fontana, D. J. *Eur. J. Pharmacol.* **1997**, *324*, 153.
3. Singh, L.; Field, M. J.; Ferris, P.; Hunter, J. C.; Oles, R. J.; Williams, R. G.; Woodruff, G. N. *Psychopharmacology* **1997**, *127*, 1. de-Paris, F.; Busnello, J. V.; Vianna, M. R. M.; Salgueiro, J. B.; Quevedo, J.; Izquierdo, I.; Kapczinski, F. *Beh. Pharmacol.* **2000**, *11*, 169.
4. Rice, A. S. C.; Maton, S. *Pain* **2001**, *94*, 215.
5. Lannesu, C.; Green, A.; Hirst, W. D.; Wise, A.; Brown, J. T.; Donnier, E.; Charles, K. J.; Wood, M.; Davies, C. H.; Pangalos, M. N. *Neuropharmacology* **2001**, *41*, 965.
6. Gee, N. S.; Brown, J. P.; Dissanayake, V. U. K.; Offord, J.; Thurlow, R.; Woodruff, G. N. *J. Biol. Chem.* **1996**, *271*, 5768.
7. Field, M. J.; Hughes, J.; Singh, L. *British J. Pharmacol.* **2000**, *131*, 282.
8. Receveur, J.-M.; Bryans, J. S.; Field, M. J.; Singh, L.; Horwell, D. C. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2329. Bryans, J. S.; Davies, N.; Gee, N. S.; Dissanayake, V. U. K.; Ratcliffe, G. S.; Horwell, D. C.; Kneen, C. O.; Morrell, A. I.; Oles, R. J.; O'Toole, J. C.; Perkins, G. M. *J. Med. Chem.* **1998**, *41*, 1838.
9. All intermediates and target compounds provided satisfactory ^1H and LCMS spectra, and elemental analysis.
10. Tanaka, T.; Oba, T.; Okamura, N.; Watanabe, K.; Kurozumi, S.; Naruchi, T. *Synth. Comm.* **1980**, *10*, 773.
11. Duus, F. *Tetrahedron* **1976**, *32*, 2817.
12. Lapinski, L.; Fulara, J.; Czerminski, R.; Nowak, M. J. *Spectrochimica Acta* **1990**, *46A*, 1087.
13. [^3H]-gabapentin binding assay was performed with A710 cell membranes and was adopted from the method previously described by Sunam-Chauhan et al., 1993: Suman-Chauhan, N.; Webdale, L.; Hill, D. R.; Woodruff, G. N. *Eur. J. Pharmacol.* **1993**, *244*, 293.
14. For rat pharmacokinetic studies, male Sprague–Dawley rats (300–400 g) were dosed at 2, 10, and 20 mg/kg iv, p.o., and ip respectively. Vehicle for the studies was saline. Heparinized blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, and 8 h. Plasma was obtained by centrifugation and stored at −80°C until analysis.
15. Kim, S. H.; Chung, J. M. *Pain* **1992**, *50*, 355.