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N-Acridin-9-yl-butane-1,4-diamine derivatives: high-affinity ligands of the $\alpha_2\delta$ subunit of voltage gated calcium channels

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Abstract—A series of *N*-acridin-9-yl-butane-1,4-diamines were found to be high-affinity ligands of the $\alpha_2\delta$ subunit of voltage gated calcium channels. The SAR studies of butane-1,4-diamine side chain resulted in the identification of compound **10** ($IC_{50}=9$ nM), which is more potent than gabapentin ($IC_{50}=27$ nM). Partial saturation of the acridine ring was also pursued and provided a compound with higher binding affinity than **1**.
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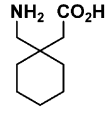
1. Introduction

Gabapentin (Neurontin®), [1-(aminomethyl)cyclohexyl]-acetic acid, is an anticonvulsant agent, and has been shown to be effective against neuropathic pain both in animal models and in human.[1–5] The exact mechanism of action of gabapentin in these therapeutic areas, however, is still unclear. Recently, a high affinity [^{3}H]gabapentin-binding protein has been identified as an $\alpha_2\delta$ subunit of a voltage gated calcium channel, and this subunit has been suggested to play an important role in the pharmacological actions of gabapentin.[6]

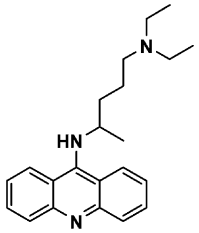
Our goal was to discover novel gabapentin-mimetic compounds, with which we can better understand the mechanism of the pharmacological actions of gabapentin and thereby develop more effective drugs. A high-throughput screening campaign of [^{3}H]gabapentin binding in human brain membrane (A710 membrane)[7] identified compound **1** as a high-affinity ligand of the $\alpha_2\delta$ subunit of a voltage gated calcium channel ($IC_{50}=220$ nM). Herein we report SAR studies on **1** and the discovery of highly potent ligands of the $\alpha_2\delta$ subunit of voltage gated calcium channels.

2. N^4-Acridin-9-yl-N^1,N^1-diethyl-1-alkylbutane-1,4-diamines

First, we investigated the binding affinity of the enantiomers of **1**, and found that the (*S*)-enantiomer **2** is the active component ($IC_{50}=110$ nM, Table 1). The stark contrast in binding affinity between **2** and **3** suggests that the methyl group of **1** is proximal to a binding pocket of the protein, thereby the protein can differentiate the (*S*) and (*R*) methyl groups. Secondly, the desmethyl analogue **4** is inactive whereas the *gem*-dimethyl analogue **5** is almost as active as **2**. Taken together these results strongly suggest a specific interaction between the ligands in this position and the protein.



gabapentin



1

We next sought the optimal length of the side chain. Preliminary SAR studies showed that all three nitrogen

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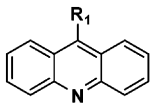
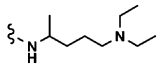
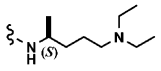
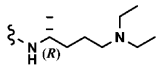
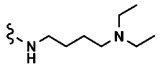
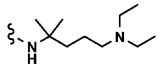
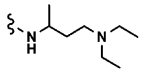
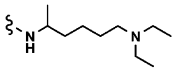
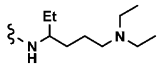
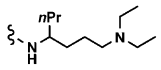
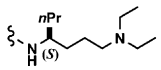
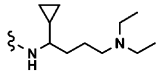
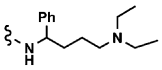
Table 1. $\alpha_2\delta$ Binding affinity of N^4-acridin-9-yl-N^1,N^1-diethyl-1-alkylbutane-1,4-diamines



Compd	R_1	$\alpha_2\delta$ Binding IC_{50} (nM)
1		220
2		110
3		11,000
4		13,000
5		270
6		> 10,000
7		6000
8		38
9		18
10		9
11		52
12		1100

atoms of **1** are essential for high binding affinity (data not shown). Thus, spacers with different numbers of carbon atoms between the two nitrogen atoms of the side chain were synthesized. With the crucial methyl group in place, neither the three carbon spacer analogue **6** nor the five carbon spacer analogue **7** showed binding activity.

SAR studies on the C4 center of the side chain were then explored, keeping the optimal four carbon spacer. The ethyl analogue **8** is five times as potent as **1** and the propyl analogue **9** is more than ten times as potent as **1** ($IC_{50} = 18$ nM). The cyclopropyl analogue **11** is comparable to **8** but the phenyl analogue **12** is less potent than **1**. As expected from the potency pattern of enantiomers **2** and **3**, the (*S*)-enantiomer **10** was indeed the active enantiomer ($IC_{50} = 9$ nM).

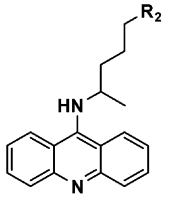
2.1. N^4-Acridin-9-yl-N^1,N^1-dialkylpentane-1,4-diamines

Pharmacokinetic studies showed rapid clearance of **1** in rats. Both des-ethyl and bis-des-ethyl analogues of **1** were observed in rat liver microsomes, which presumably accounts for the rapid clearance of **1** in this species. To improve the poor pharmacokinetic properties of **1**, SAR studies on the terminal diethylamino group were carried out. Both acyclic and cyclic amine analogues with various steric and electronic demand at the C1 position of the side chain were synthesized (Table 2). These studies revealed a very tight SAR; minor modification at the C1 center of the side chain furnished completely inactive analogues. Neither bulkier alkyl amine analogues (**13** and **14**) nor cyclic amine analogues (**15**–**18**) showed high binding affinity to the $\alpha_2\delta$ subunit of voltage gated calcium channels. Analogues having aromatic heterocyclic amino groups such as imidazole, triazole and tetrazole at the C1 center of the side chain were also inactive in the binding assay (data not shown).

2.2. Partial saturation of N^4-acridin-9-yl-N^1,N^1-diethyl-pentane-1,4-diamines

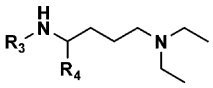
Since planar aromatic dyes such as 9-aminoacridine have been known to be mutagenic,[8] potential genetoxicity of these compounds was one of our initial concerns. To address this issue, we pursued analogues having different ring structures. The quinoline and tetrahydroquinoline analogues **19** and **20** turned out to be much less potent than **1** suggesting that a tricyclic ring skeleton is required for high binding affinity (Table 3). To avoid planarity of the acridine ring, partial saturation of the aromatic ring system was pursued. Although the tetrahydro analogue **21** is not as potent as **1**, its potency indicates that there is room for further SAR studies on the tricyclic ring. Interestingly, further saturation of the ring to the octahydro tricyclic ring system provided a more potent compound (**22**, $IC_{50} = 160$ nM) than **1**. The potency trend of enantiomer pairs also applied to **22**; the (*S*)-enantiomer **23** has much higher binding affinity than the (*R*)-enantiomer **24** ($IC_{50} = 43$ nM and 760 nM respectively).

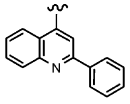
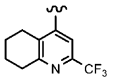
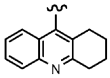
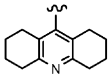
Table 2. $\alpha_2\delta$ Binding affinity of N^4-acridin-9-yl-N^1,N^1-dialkyl-pentane-1,4-diamines



Compd	R_2	$\alpha_2\delta$ Binding IC_{50} (nM)
13	–NH*i*Pr	5100
14	–NH*i*Pr$_2$	> 10,000
15		9900
16		> 10,000
17		> 10,000
18		5900

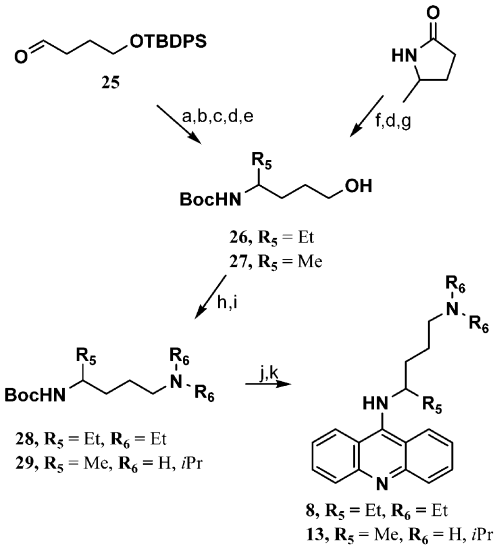
Table 3. $\alpha_2\delta$ Binding affinity of partially saturated N^4-acridin-9-yl-N^1,N^1-diethylpentane-1,4-diamines



Compd	R_3	R_4	$\alpha_2\delta$ Binding IC_{50} (nM)
19		*rac*-CH_3	960
20		*rac*-CH_3	>10,000
21		*rac*-CH_3	420
22		*rac*-CH_3	160
23		(*S*)-CH_3	43
24		(*R*)-CH_3	760

3. Chemistry

All the analogues of **1** herein studied were synthesized in a convergent fashion via coupling 9-chloroacridine[9] with the relevant side chains as the last step. For example, the synthesis of ethyl analogue **8** is depicted in Scheme 1. Aldehyde **25** was prepared by following the known literature procedure[10] on a large scale and coupled to the ethyl Grignard reagent. The resultant secondary alcohol was converted to the corresponding amine via the phthalimide and the amine was protected with a Boc group to provide **26**. After deprotection of the TBDPS group, the diethylamino moiety was introduced at the C1 center by displacement of the corresponding mesylate with diethylamine to afford **28**. Deprotection of the Boc group followed by coupling (PhOH, TEA, 120 °C)[11] to 9-chloroacridine proceeded smoothly to provide **8** in high yield.



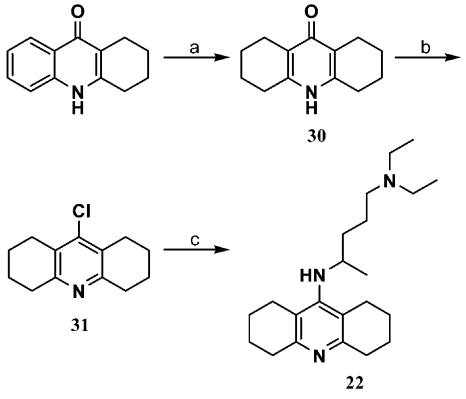
Scheme 1. (a) EtMgBr, THF; (b) phthalimide, PPh_3, DEAD, THF; (c) N_2H_4, EtOH; (d) Boc_2O, TEA, CH_2Cl_2; (e) TBAF, THF; (f) 6 N HCl, reflux; (g) $ClCO_2Et$, NMM, THF; $NaBH_4$, MeOH; (h) MsCl, TEA, THF; (i) Et_2NH or $iPrNH_2$, CH_3CN, 50 °C; (j) 4 N HCl, 1,4-dioxane; (k) 9-chloroacridine, PhOH, TEA, 120 °C.

The synthesis of side chains with various amino groups at the C1 center (see Table 2) started from 5-methyl-2-pyrrolidinone. Hydrolysis followed by Boc protection and reduction of the resultant amino acid provided alcohol **27**. By following the same protocol utilized for the preparation of **8**, the isopropylamine analogue **13** was prepared in good overall yield.

As an example of the synthesis of partially saturated analogues (see Table 3), the synthesis of octahydro analogue **22** is shown in Scheme 2. Reduction of 1,2,3,4-tetrahydro-9-acridanone followed by chlorination provided 9-chloro-1,2,3,4,5,6,7,8-octahydroacridine (**31**). Buchwald amination of **31** proceeded smoothly to provide the octahydro analogue **22** in good yield.[12]

4. [^{13}H]-10: A potent and selective radioligand

Compound **10**, the most potent ligand identified in this study, was radiolabeled to measure specific versus non-specific binding and to provide a tool for in vitro binding assays. Binding studies with [^{3}H]-**10** using gabapentin as cold displacer showed high specific binding of [^{3}H]-**10** to the $\alpha 2\delta$ subunit of human calcium channels (about 90% of total binding, Fig. 1). This result supports the use of [^{3}H]-**10** as a potent and selective radioligand for in vitro binding assays.



Scheme 2. (a) H_2, PtO_2, HCl; (b) $POCl_3$, reflux; (c) 2-amino-5-diethylaminopentane, $Pd(OAc)_2$, NaOtBu, biphenyl-2-yl-di-*tert*-butyl-phosphane, toluene.

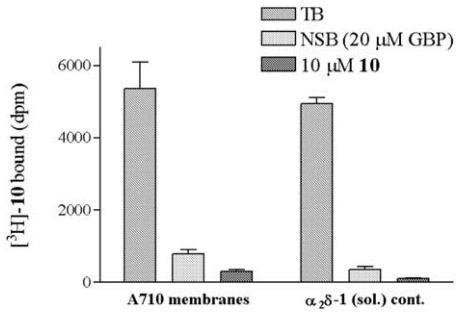


Figure 1. Specific binding of [^{3}H]-**10** to $\alpha_2\delta$ subunit of human voltage gated calcium channels.[7]

5. Conclusion

Ligand **10** which binds potently ($IC_{50} = 9$ nM) to the $\alpha 2\delta$ subunit of calcium channels was identified through optimization of the side chain of the lead compound **1**. To the best of our knowledge, **10** is the first high affinity, non amino acid gabapentin-mimetic ligand reported to date. A potent octahydroacridine was also prepared. Binding studies with [^{3}H]-**10** validated it as a specific radioligand.

Acknowledgements

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References and notes

1. Taylor, C. P. The role of gabapentin. In *New Trends in Epilepsy Management*; Chadwick, D., Ed.; Royal Society of Medicine Services Ltd: London, 1993; pp 13–40.
2. Rosner, H.; Rubin, L.; Kestenbaum, A. *Clin. J. Pain* **1996**, *12*, 56.
3. Singh, L.; Field, M. J.; Ferris, P.; Hunter, J. C.; Oles, R. J.; Williams, R. G. *Psychopharmacology (Berlin)* **1996**, *127*, 1.
4. Backonja, M.; Glanzman, R. L. *Clin. Ther.* **2003**, *25*, 81.
5. Field, M. J.; Hughes, J.; Singh, L. *Br. J. Pharmacol.* **2000**, *131*, 282.
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. [^{3}H]Gabapentin binding assay in human brain membrane (A710 membrane) was adopted from the method in the literature: Suman-Chauhan, N.; Webdale, L.; Hill, D. R.; Woodruff, G. N. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1993**, *244*, 293.
8. Topal, M. D. *Biochemistry* **1984**, *23*, 2367.
9. Cain, B. F.; Seelye, R. N.; Atwell, G. J. *J. Med. Chem.* **1974**, *17*, 922.
10. Spino, C.; Barriault, N. *J. Org. Chem.* **1999**, *64*, 5292.
11. Phanstiel, O., IV; Price, H. L.; Wang, L.; Juusola, J.; Kline, M.; Shah, S. M. *J. Org. Chem.* **2000**, *65*, 5590.
12. Wolfe, J. P.; Tomori, H.; Sadighi, J. P.; Yin, J.; Buchwald, S. L. *J. Org. Chem.* **2000**, *65*, 1158.