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Bioorganic & Medicinal Chemistry Letters 14 (2004) 2463–2467
Bioorganic & Medicinal Chemistry Letters

Identification and synthesis of [1,2,4]triazolo[3,4-*a*]phthalazine derivatives as high-affinity ligands to the $\alpha_2\delta$-1 subunit of voltage gated calcium channel

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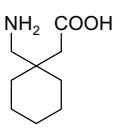
Received 3 February 2004; revised 26 February 2004; accepted 3 March 2004

Abstract—We have identified and synthesized a series of [1,2,4]triazolo[3,4-*a*]phthalazine derivatives as high-affinity ligands to $\alpha_2\delta$-1 subunit of voltage gated calcium channels. Structure–activity relationship studies directed toward improving the potency and physical properties of **2** lead to the discovery of **20** ($IC_{50} = 15$ nM) and (*S*)-**22** ($IC_{50} = 30$ nM). A potent and selective radioligand, [^{3}H]-(*S*)-**22** was also synthesized to demonstrate that this ligand binds to the same site as gabapentin.

1. Introduction

Gabapentin (1-(aminomethyl)cyclohexane acetic acid; Neurontin®) **1** is an anticonvulsant[1] that is also efficacious for the treatment of neuropathic pain in human and animal models.[2] Recently, gabapentin has been shown to bind with high affinity to the $\alpha_2\delta$-1 subunit of voltage gated calcium channel[3] ($IC_{50} = 30$ nM).[4] Although the mechanism of action is not well understood, gabapentin's pharmacological actions have been hypothesized to be mediated through this high affinity binding site.



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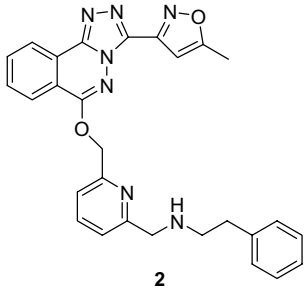

2

We sought to discover novel small molecule lead structures with high affinity binding to $\alpha_2\delta$-1 subunit. The initial lead, [1,2,4]triazolo[3,4-*a*]phthalazine **2** with a binding affinity for $\alpha_2\delta$-1 of $IC_{50} = 220$ nM was identified by a high throughput screen. Herein, we report the identification and synthesis of [1,2,4]triazolo[3,4-*a*]phthalazine derivatives as high-affinity ligands to the $\alpha_2\delta$-1 subunit of voltage gated calcium channel. Our primary goal was to optimize the binding affinity of these ligands through structure–activity relationships (SAR) focusing mainly on modifications to the methyl isoxazole and phenethylamine moieties.

2. Biological results

Meager physical properties such as poor solubility led to the immediate replacement of the methyl isoxazole unit in **2** with more soluble groups (Table 1). In general, replacement of the methyl isoxazole with a variety of alkyl groups resulted in only modest changes in binding affinity for the $\alpha_2\delta$-1 subunit. However, replacement of methyl isoxazole by trifluoromethyl **5** produced a 3-fold increase in potency ($IC_{50} = 72$ vs 220 nM) and also

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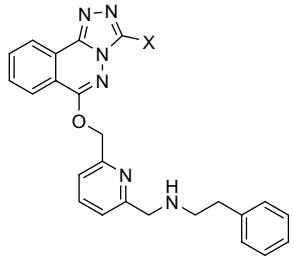
doi:10.1016/j.bmcl.2004.03.008

Table 1. $\alpha_2\delta$-1 Binding affinities for isoxazole replacements



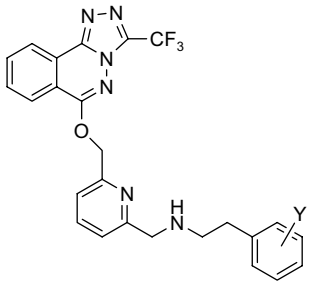
Compds	X	$\alpha_2\delta$-1 binding IC_{50} (nM)
2		220
3	H	230
4	Me	145
5	CF_3	72
6	SMe	194
7	Et	241
8	CH_2OCH_3	131
9		272
10	*i*-Pr	342
11		145
12		195

provided improved solubility. Therefore our strategy consisted of maintaining the 3-trifluoromethyl functionality in the [1,2,4]triazolo[3,4-*a*]phthalazine, while SAR was conducted on the phenethylamine portion of the molecule.

Having discovered the trifluoromethyl group as a replacement for methyl isoxazole, we turned our attention to substituting methoxy, fluorine, and methyl groups around the phenyl ring (Table 2). From the structure–activity trends found in compounds **13**–**21**, it was apparent that the 3-substituted phenyl rings were consistently more potent than the corresponding 2- or 4-substitutions. The most potent compound in this series was 3-methyl-phenyl-[1,2,4]triazolo[3,4-*a*]phthalazine **20** ($IC_{50} = 15$ nM) showing a 5-fold increase in potency over unsubstituted phenyl **5** and a 14-fold increase over the initial methyl isoxazole lead **2**.

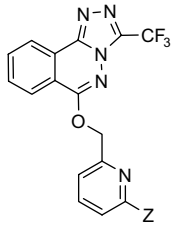
As part of our efforts to improve metabolic stability of the amine in the scaffold, we incorporated alkyl groups on the linking tether between the pyridine and phenyl moiety (Table 3). These changes resulted in the identification of another high affinity analogue, *rac*-**22** ($IC_{50} = 36$ nM). To determine the influence of the chiral center on the biological activity, the enantiomers of *rac*-**22** were synthesized. As shown in Table 3, only the (*S*)-**22** ($IC_{50} = 30$ nM) showed binding affinity while (*R*)-**22** was essentially inactive. Exchanging the methyl group in *rac*-**22** for other alkyl groups such as *rac*-**23** and *rac*-**24**

Table 2. $\alpha_2\delta$-1 Binding affinities for substitutions on phenyl ring



Compds	Y	$\alpha_2\delta$-1 binding IC_{50} (nM)
13	2-OMe	3163
14	3-OMe	116
15	4-OMe	829
16	2-F	291
17	3-F	161
18	4-F	630
19	2-Me	448
20	3-Me	15
21	4-Me	260

Table 3. $\alpha_2\delta$-1 Binding affinities for modifications to linking tether



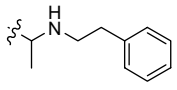
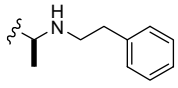
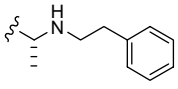
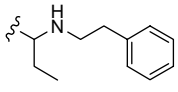
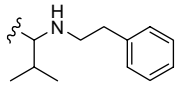
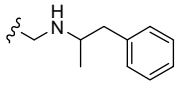
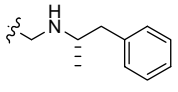
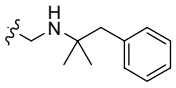
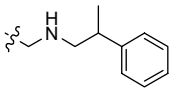
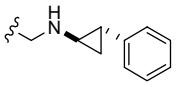
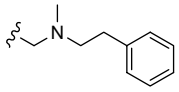
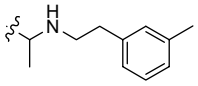
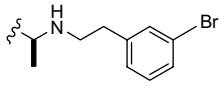
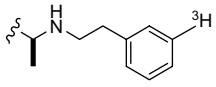
Compds	Z	$\alpha_2\delta$-1 binding IC_{50} (nM)
rac-**22**		36
(*S*)-**22**		30
(*R*)-**22**		10,000
rac-**23**		183
rac-**24**		174
rac-**25**		1369
(*S*)-**25**		700

Table 3 (*continued*)

Compds	Z	$\alpha_2\delta$-1 binding IC_{50} (nM)
26		10,000
rac-**27**		198
rac-**28**		229
29		10,000
rac-**30**		29
(*S*)-**31**		28
[^{3}H]-(*S*)-**22**		30

led to significant decreases in binding affinity. Unfortunately, synthesis of a hybrid structure *rac*-**30** ($IC_{50} = 29$ nM), afforded similar potency to that of the parent structures **20** and *rac*-**22**.

To rigorously establish that binding affinities of the compounds presented were specific for $\alpha_2\delta$-1, we prepared [^{3}H]-(*S*)-**22** from the corresponding bromide (*S*)-**31** by hydrogenation (Table 3). [^{3}H]-(*S*)-**22** showed high specific binding to A710 membranes[4] and soluble human $\alpha_2\delta$-1 ([^{3}H]-(*S*)-**22**, $IC_{50} = 30$ nM) when using both gabapentin and (*S*)-**22** as a cold displacer (Fig. 1). This result supports the use of [^{3}H]-(*S*)-**22** as a potent radioligand for in vitro binding assays and demonstrated that this ligand binds to the same site as gabapentin.

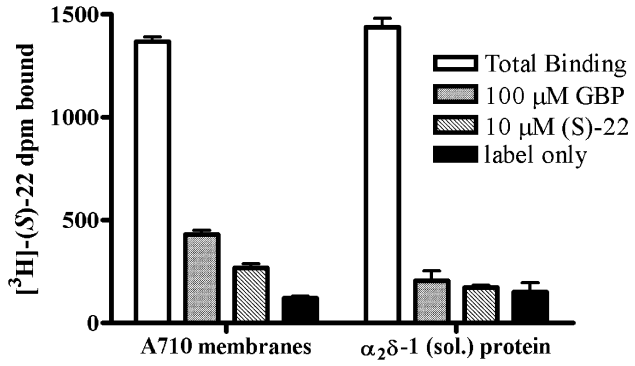
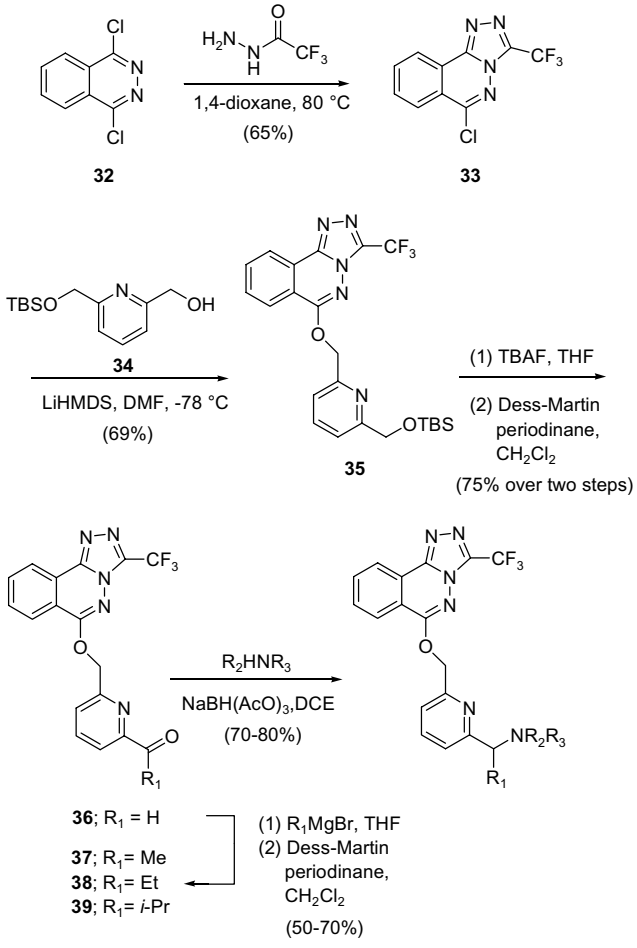


Figure 1. Specific binding of [^{3}H]-(*S*)-**22** to $\alpha_2\delta$-1 subunit of human voltage gated calcium channels.[5]

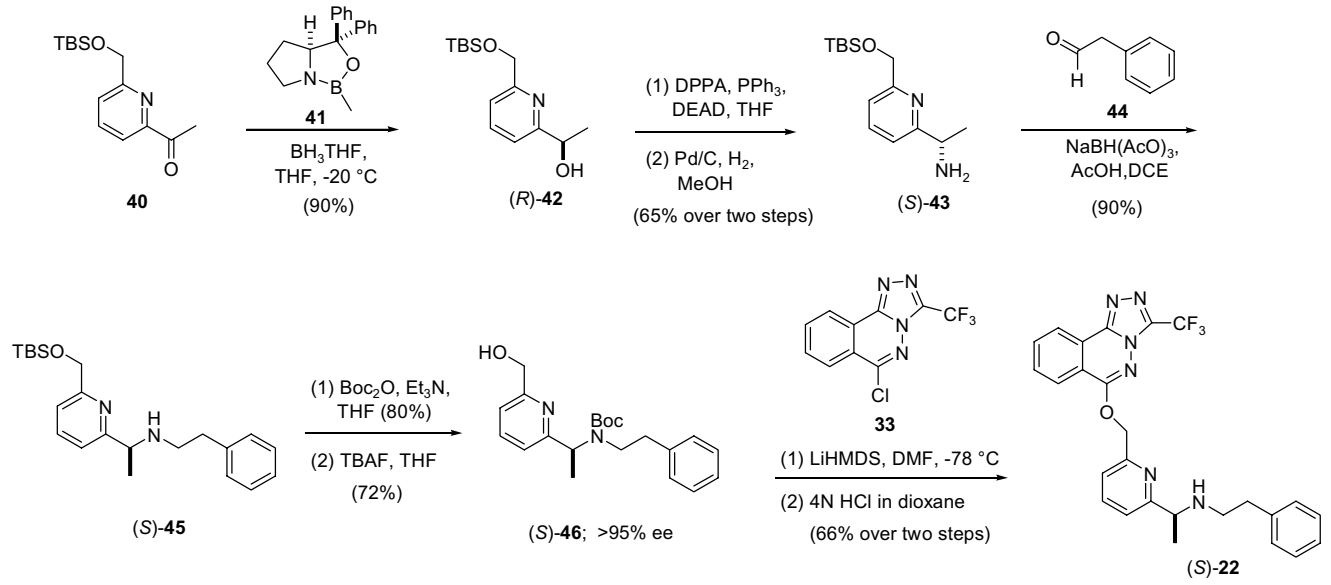
3. Chemistry

The synthesis began with commercially available 1,4-dichlorophthalazine **32**,[6] which was transformed to 6-chloro-3-trifluoromethyl-[1,2,4]triazolo[3,4-*a*]phthalazine **33** using an improved version of a procedure developed earlier (Scheme 1).[7] Exposure of 6-chloro-3-trifluoromethyl-[1,2,4]triazolo[3,4-*a*]phthalazine **33** and pyridine **34**[8] to lithium bis(trimethylsilyl)amide in DMF at −78 °C delivered the coupled pyridal-[1,2,4]triazolo[3,4-*a*]phthalazine **35** in 69% yield.[9] Cleavage of the silyl ether in the presence of TBAF and oxidation of the resulting alcohol with Dess–Martin periodinane[10] provided aldehyde **36** in 75% yield over two steps. Reductive amination of the aldehyde **36** in the presence of the corresponding amine (R_2HNR_3) and $NaBH(AcO)_3$ provided **5**, **13**–**21**, and **25**–**29** in 70–80% yield.[11] A similar reductive amination protocol was used to prepare *rac*-**22**–*rac*-**24** and *rac*-**30** from ketones **37**–**39**.

The synthetic approach to enantiomerically enriched [1,2,4]triazolo[3,4-*a*]phthalazine (*S*)-**22** is detailed in Scheme 2. Reduction of ketone **40**[12] by reaction with 0.7 equiv of (*S*)-oxazaborolidine **41** and 1.1 equiv of BH_3·THF at −20 °C provided enantioenriched alcohol (*R*)-**42**.[13,14] Mitsunobu reaction of the alcohol (*R*)-**42**



Scheme 1. General synthesis of [1,2,4]triazolo[3,4-*a*]phthalazine derivatives.



Scheme 2. Synthesis of enantioenriched [1,2,4]triazolo[3,4-*a*]phthalazine derivative (*S*)-**22**.

followed by reduction of the resulting azide afforded amine (*S*)-**43** in 65% yield over two steps.[15] Reductive amination of (*S*)-**43** and phenylacetylaldehyde **44** by treatment of $NaBH(AcO)_3$ in dichloroethane provided (*S*)-**45** in 90% yield.[9] The secondary amine of (*S*)-**45** was protected with di-*tert*-butoxycarbonate ($(Boc)_2O$) to afford the carbamate in 80% yield. Cleavage of the *tert*-butyldimethylsilyl ether in the presence of TBAF provided alcohol (*S*)-**46** in 72% yield, >95% ee by chiral HPLC analysis.[16] Coupling of (*S*)-**46** and **33** in the presence of lithium bis(trimethylsilyl)amide in DMF at −78 °C followed by exposure of the product to 4 N HCl in dioxane cleaved the *tert*-butoxycarbonyl group to provide (*S*)-**22** in 66% yield over two steps. Synthesis of (*R*)-**22** was achieved by replacing (*S*)-oxazaborolidine **41** with (*R*)-oxazaborolidine in the initial reduction step of Scheme 2. In addition, (*S*)-**31** could be easily accessed by replacing phenylacetylaldehyde **44** with 3-bromophenylacetylaldehyde in Scheme 2.[17]

4. Conclusion

In summary, we have discovered several [1,2,4]triazolo[3,4-*a*]phthalazine derivatives such as (*S*)-**22** and **20** that are high-affinity ligands to $\alpha_2\delta$-1 and are structurally distinct from gabapentin. Use of the [^{3}H]-(*S*)-**22** in binding studies revealed it binds with high affinity to soluble human $\alpha_2\delta$-1 and is displaced by cold gabapentin and cold (*S*)-**22**. The ligands described herein will be used as tools to better understand the role of $\alpha_2\delta$-1 in various gabapentin mediated mechanisms.

Acknowledgements

The authors would like to thank William Bray for analytical support.

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