Bioorganic & Medicinal Chemistry Letters 21 (2011) 6582–6585

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of a novel potent $GABA_B$ receptor agonist

Feng Xu, Ge Peng, Thu Phan, Usha Dilip, Jian Lu Chen, Tania Chernov-Rogan, Xuexiang Zhang, Kent Grindstaff, Thamil Annamalai, Kerry Koller, Mark A. Gallop, David J. Wustrow *

XenoPort Inc., 3410 Central Expressway, Santa Clara, CA 95051, USA

ARTICLE INFO

Article history:
Received 28 June 2011
Revised 29 July 2011
Accepted 1 August 2011
Available online 17 August 2011

Keywords:
GABAB
Baclofen
SAR
Palladium
Boronate
Functional potency
Pyridylmethyl ether

ABSTRACT

Structure–activity studies have led to a discovery of 3-(4-pyridyl)methyl ether derivative **9d** that has 25- to 50-fold greater functional potency than R-baclofen at human and rodent $GABA_B$ receptors in vitro. Mouse hypothermia studies confirm that this compound crosses the blood–brain barrier and is approximately 50-fold more potent after systemic administration.

Molecules that positively modulate GABA receptors continue to be of great medicinal interest for a number of disease states.[1] The prototypical $GABA_B$ agonist baclofen **1** (Fig. 1) is widely used in the treatment of spasticity,[2] and has shown evidence for potential clinical utility in treating other conditions including drug addiction and alcoholism,[3] gastroesophageal reflux disease (GERD),[4] cancer pain[5] and overactive bladder.[6] The $GABA_B$ activity of racemic baclofen is known to reside primarily in the *R*-(−)-enantiomer **2**. Currently *R*-(−)-baclofen is under development for the treatment of behavioral symptoms of Fragile X Disorder. A prodrug of *R*-(−)-baclofen, XP19986[7] **3**, is under clinical development as a potential improved treatment for spasticity.

Since the initial synthesis of baclofen more than 40 years ago,[8] relatively few studies of baclofen analogs exploring alternative aromatic substitution have been published.[9] We undertook a structure–activity study in which a broad range of substituents were introduced to the aromatic ring of **2** in order to understand their effects on $GABA_B$ receptor potency. In this paper, we report discovery of a novel series of compounds that show improved $GABA_B$ receptor potency in vitro and in vivo as compared to **2**.

Synthesis began with the readily available lactam **4** which can be prepared in high enantiomeric purity following a variety of literature methods.[10–13] Nitration of lactam **4** followed by reduction produced aniline **5** (Scheme 1). Conversion of the anilino group to an iodide using Sandmeyer chemistry followed by Pd-catalyzed substitution led to the dioxaborolane derivative **6**. Oxidation of the borolane with hydrogen peroxide afforded phenol **7**, which could be converted to the desired products by two alternate methods. Phenol ethers **8** could be prepared via a modified Mitsunobu reaction procedure. The target baclofen derivatives were prepared

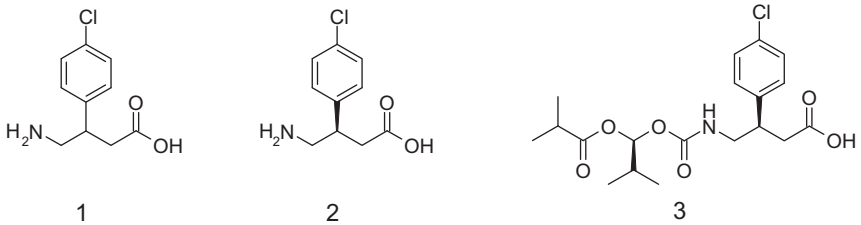
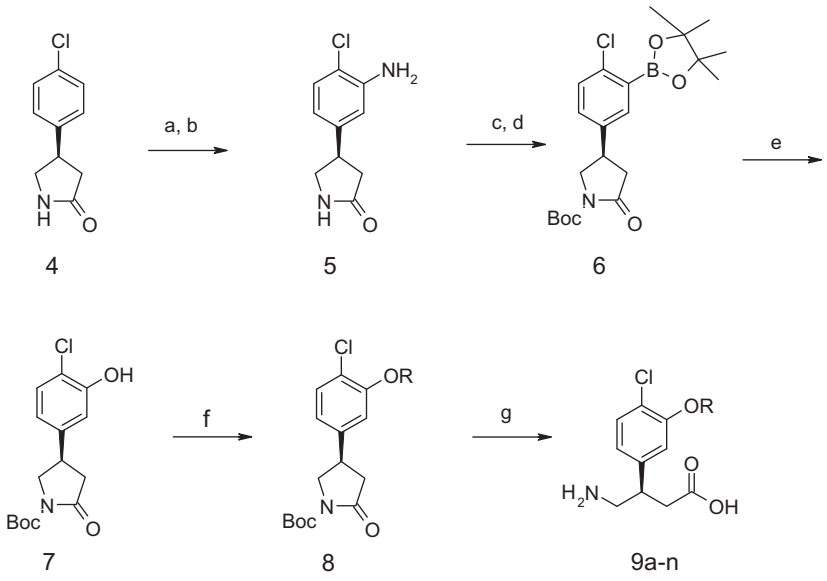


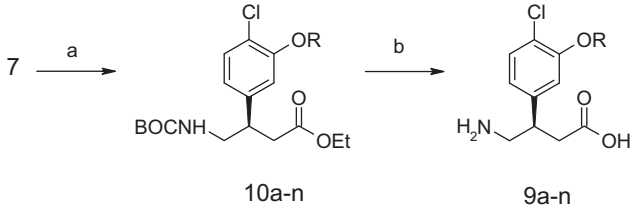
Figure 1.

* Corresponding author.
E-mail address: Dave.Wustrow@XenoPort.com (D.J. Wustrow).

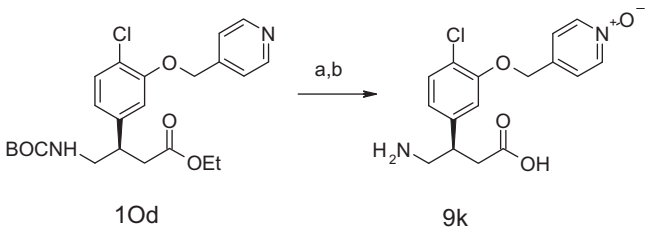
0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.08.006



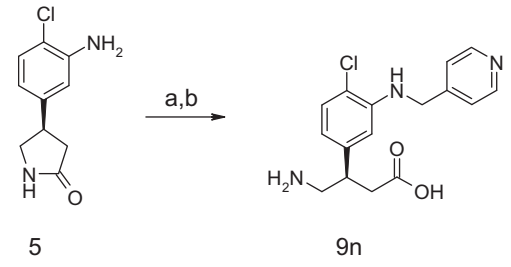
Scheme 1. Reagents and conditions: (a) Guanidine nitrate, H_2SO_4 concd, 0 °C; (b) Fe, acetic acid, 50 °C; (c) $NaNO_2$, KI, 12 N HCl, 0 °C 55% over three steps; (d) $PdCl_2(dppf)_2$, octamethyl 2,2-bis-1,3,2-dioxaborolane, KOAc, DCM–dioxane 43%; (e) H_2O_2, DCM 82%; (f) alcohol, DIAD, PPh_3 DCM; (g) (i) NaOH, (ii) 6 N HCl, 90 °C or TFA DCM.



Scheme 2. Reagents and conditions: (a) Cs_2CO_3, alkyl bromide, EtOH, 70 °C; (b) 6 N HCl, 90 °C. Overall yield 23–50%.



Scheme 3. Reagents and conditions: (a) 35% H_2O_2; 6 N HCl, reflux 7 h, HPLC 8% yield.



Scheme 4. Reagents and conditions: (a) 4-pyridinecarboxaldehyde, 2-picoline borane, MeOH–AcOH 10:1; (b) 6 N HCl, acetonitrile 90 °C 3 h. HPLC 11% yield.

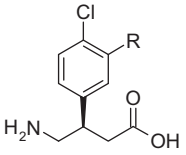
by hydrolysis and then isolated following HPLC purification in low to moderate yields (Method A). Alternatively phenol **7** could be reacted with alkyl halides in ethanol in the presence of Cs_2CO_3. In addition to facilitating phenolic alkylation, the ethoxide formed under these conditions also opened the lactam imide giving the BOC-protected ester-ethers **10**. BOC-esters **10** were converted to the target compounds **9** via acidic hydrolysis (Method B, Scheme 2).

The pyridine N-oxide derivative **9k** was prepared through reaction of intermediate **10d** with concentrated peroxide followed by acid hydrolysis and HPLC purification (Scheme 3). The substituted aniline derivative **9n** could be prepared by reacting aniline **5** with 4-pyridinecarboxaldehyde under reductive amination conditions, followed by hydrolysis in aqueous acid (Scheme 4). All target compounds were isolated by semi-preparative reversed-phase HPLC using a C_{18} stationary phase column and eluting with water–acetonitrile gradients containing 0.5% formic acid. ES-MS was employed during fraction collection and was used to guide isolation of the appropriate sample-containing fractions. Purified compounds were isolated by lyophilization from aqueous HCl and characterized using mass spectrometry, ^1H NMR and HPLC to assess structure and purity.

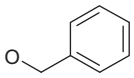
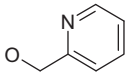
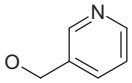
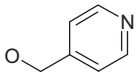
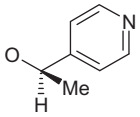
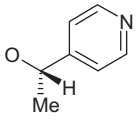
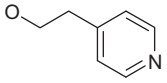
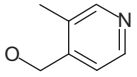
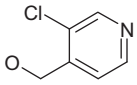
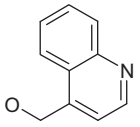
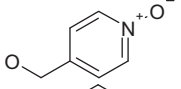
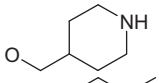
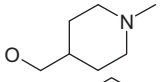
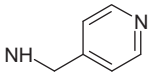
Compounds were assessed for their in vitro potency as agonists at the human $GABA_B$ receptor. Human embryonic kidney (HEK) cells constitutively expressing $G_{q/i}$ proteins were transfected with human $GABA_{B1a}$ and $GABA_{B2}$ receptors under control of a tetracycline-inducible promoter. Activation of the $GABA_B$ receptor complex was quantified through measurement of the increased levels of cytoplasmic Ca^{2+}, released from intracellular stores, and detected with a Ca^{2+} sensitive fluorescent probe. The concentration of half-maximal stimulation was calculated and expressed as the pEC_{50} (Table 1).

The active R isomer of baclofen **2** was shown to have a pEC_{50} of 7.1 at human $GABA_B$ receptors (with the V_{max} defined as 100%). pEC_{50} values for test compounds were calculated from duplicate determinations. Substitution of the aromatic ring of R-baclofen with a benzyl ether (**9a**) or 2-pyridylmethyl (**9b**) resulted in decreased agonist potency at the $GABA_B$ receptor. The loss in potency was partially reversed by the replacement of the phenyl ring of **9a** with a 3-pyridyl ring (compound **9c**). Introduction of the 4-pyridyl substituent in **9d** resulted in a 2.4 order of magnitude increase in potency. Indeed compound **9d** was more than one order of magnitude (25-fold) more potent than R-baclofen **2** as an agonist at the human $GABA_B$ receptor in this assay.

Table 1
Synthesis method and in vitro functional potency of compounds at human $GABA_B$ receptor



2, 9a-n

No.	Method	R	h-$GABA_B$ agonist pEC_{50}
2	—	H	7.1
9a	A		6.1
9b	B		4.0
9c	B		6.3
9d	B		8.5
9e	A		7.3
9f	A		5.3
9g	A		7.4
9h	B		7.2
9i	B		6.6
9j	B		3.9
9k	—		6.7
9l	B		<3
9m	A		3.6
9n	—		6.8

A series of analogs of **9d** were prepared and evaluated. The α-methyl-substituted derivatives of **9d** show a significant difference in potency between the stereoisomers **9e** and **9f**. The introduction of an ethyl spacer between the pyridine and oxygen functionality in **9g** resulted in a compound slightly more potent than baclofen, but reduced in potency compared to **9d**. Substitution of the pyridine ring as exemplified by **9h–j** led to decreases in potency compared to **9d**. The potency decrease was accentuated for electron withdrawing and aromatic ring substituents. Similarly pyridine N-oxide **9k** also possessed reduced agonist potency at the $GABA_B$ receptor. The saturated piperidines **9l** and **9m** had little or no agonist activity at the human $GABA_B$ receptor. Replacement of the ether oxygen linkage of **9d** with nitrogen functionality resulted in aniline **9n**, which had decreased agonist potency.

To further characterize the $GABA_B$ agonist activity of **9d**, it was compared to R-baclofen **2** for its ability to induce $GABA_B$ receptor-mediated G-protein activated inwardly rectifying K^+ (GIRK) currents. Patch clamp electrophysiological experiments were performed in rat hippocampal slices containing pyramidal cell CA1 neurons as previously described.[14,15] Currents were measured in the presence of varying concentrations of R-baclofen **2** and **9d**. As shown in Figure 2 compound **9d** stimulated GIRK currents at approximately 60-fold lower concentrations than **2**. The magnitude of the maximal stimulation was the same as that observed with R-baclofen (**2**).

The stimulation of GIRK currents caused by **9d** could be completely reversed by the specific $GABA_B$ receptor antagonist CGP55845 as shown in Figure 3. Taken together these studies demonstrate that compound **9d** also has a more potent interaction than **2** with the rodent isoform of the $GABA_B$ receptor. This is further consistent with radioligand binding experiments using rat whole brain tissue which showed that compound **9d** displaced the $GABA_B$ antagonist [^{3}H]CGP54626 more potently than did **2**, with pIC_{50} values of 6.7 and 5.5, respectively.

The in vivo pharmacology of $GABA_B$ agonist **9d** was next compared with R-baclofen **2**. The mouse hypothermia model[16] was

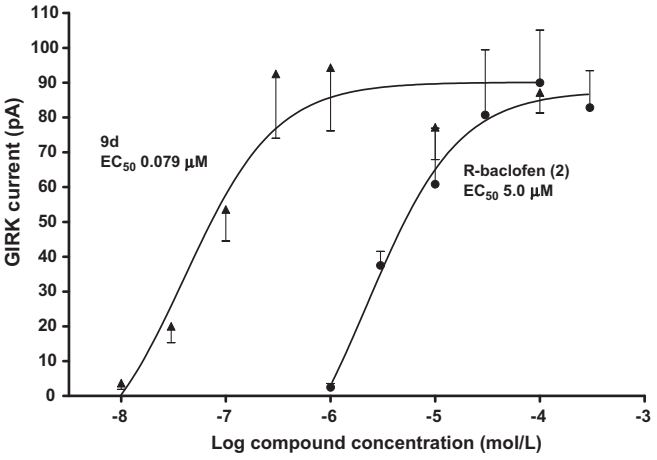


Figure 2. $GABA_B$ receptor evoked GIRK currents in rat pyramidal cell neurons.

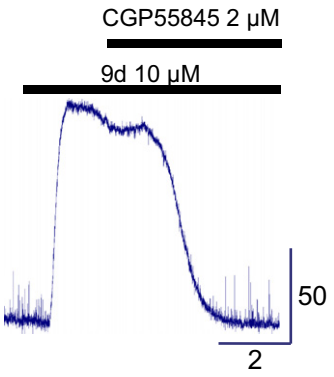
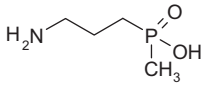
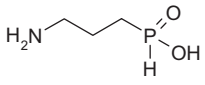
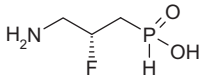


Figure 3. Reversal of GIRK current stimulation by **9d** with CGP54626.

Table 2
Mouse hypothermia data

Compound	Dose (mol/kg)	MaxTemp decrease (°C)
Vehicle	—	0
2	24	4.2
9d	0.125	0
	0.25	1
	0.4	2.4
	0.5	4.0

Table 3
Aminophosphinic $GABA_B$ agonists

Compound	Structure	h-$GABA_B$ agonist pEC_{50}
11		7.4
12		7.6
13		8.1

chosen since this assay provides a convenient readout of the centrally-mediated effects of a $GABA_B$ receptor ligand. A thermosensitive chip is implanted in the interscapular region of CD-1 mice under brief isoflurane anesthesia, and the animals are allowed to recover for at least 1 day. The animals have free access to food and water throughout the experiment. On the experimental day, the mice are placed in individual cages. Before dosing, basal temperature recordings are made using a transponder communicating with a computer for data acquisition. Test compound or controls are injected intraperitoneally at an appropriate dose. Measurements are then made at 15 min intervals post-dose up to 2 h and every 30 min thereafter to 4 h (Table 2).

In the mouse hypothermia assay both R-baclofen **2** and compound **9d** caused significant body temperature reductions. Doses of **2** and **9d** that caused a similar reduction in body temperature (4–4.2 °C) suggest that the in vivo potency of **9d** in this model is some 40–50 times that of **2**. This is consistent with the 25- to 60-fold functional potency increase observed for **9d** relative to compound **2** in vitro.

Compound **9d** was shown to be more than 10-fold more potent than R-baclofen. Previously aminophosphinic acids **11** and **12** were shown to be more potent $GABA_B$ agonists than baclofen.[17,18] More recently the 3-fluoro analogue **13** (AZD-3355) was identified as a potent $GABA_B$ agonist with restricted access to the CNS.[19] This compound has been studied clinically as a possible treatment for GERD but was recently dropped from development, in part, due to liver toxicity issues. The potency of **9d** was compared to **11**, **12** and **13** in the functional $GABA_B$ agonist assay. The pEC_{50} values were in the range of 7.4 and 8.1, respectively (Table 3). While the phosphinic analogues were more potent than R-baclofen (**2**), compound **9d** with a pEC_{50} of 8.5 is more potent than any of the phosphinic acid compounds.

In summary a series of R-baclofen analogs have been prepared that investigated the effect of substitution at the 3-position of the phenyl ring. Analogs containing a 4-pyridyl substituent appended to this position had improved in vitro potency compared to R-baclofen. The most potent of these compounds, **9d**, demonstrated in vitro $GABA_B$ agonist activity in functional assays with cloned human receptors and in a rat neuronal electrophysiological assay, appears to cross the blood–brain barrier and exhibits potent activity in vivo.

References and notes

1. Froestl, W. *Future Med. Chem.* **2011**, *3*, 163.
2. Smith, C. R.; LaRocca, N. G.; Giesser, B. S.; Scheinberg, L. C. *Neurology* **1991**, *41*, 1829.
3. Tayacke, R. J.; Lingford-Hughes, A.; Reed, L. J.; Nutt, D. J. *Adv. Pharmacol.* **2010**, *58*, 373.
4. Lidums, I.; Lehmann, A.; Checklin, H.; Dent, J.; Holloway, R. H. *Gastroenterology* **2000**, *118*, 7.
5. Yomiya, K.; Matsuo, N.; Tomiyasu, S.; Yoshimoto, T.; Tamaki, T.; Suzuki, T.; Matoba, M. *Am. J. Hosp. Palliat. Med.* **2009**, *26*, 112.
6. Taylor, M. C.; Bates, C. P. *Br. J. Urol.* **1979**, *51*, 504.
7. Lal, R.; Sukbuntherng, J.; Tai, E. H.; Upadhyay, S.; Yao, F.; Warren, M. S.; Luo, W.; Bu, L.; Nguyen, S.; Zamora, J.; Peng, G.; Dias, T.; Bao, Y.; Ludwikow, M.; Phan, T.; Scheuerman, R. A.; Yan, H.; Gao, M.; Wu, Q. Q.; Annamalai, T.; Raillard, S. P.; Koller, K.; Gallop, M. A.; Cundy, K. C. *J. Pharmacol. Exp. Ther.* **2009**, *330*, 911.
8. Keberle, H; Faigle, J. W.; Wilhelm, M. Amino acids having useful pharmacological properties. Patentschrift (Switz.), 1968.
9. Berthelot, P.; Vaccher, C.; Musadad, A.; Flouquet, N.; Debaert, M.; Luyckx, M. *J. Med. Chem.* **1987**, *30*, 743.
10. Corey, E. J.; Zhang, F.-Y. *Org. Lett.* **2000**, *2*, 4257.
11. Li, H.; Wang, Y.; Tang, L.; Deng, L. *J. Am. Chem. Soc.* **2004**, *126*, 9906.
12. Okino, T.; Hoashi, Y.; Furukawa, T.; Xu, X.; Takemoto, Y. *J. Am. Chem. Soc.* **2005**, *127*, 119.
13. Shao, C.; Yu, H.-J.; Wu, N.-Y.; Tian, P.; Wang, R.; Feng, C.-G.; Lin, G.-Q. *Org. Lett.* **2011**, *13*, 788.
14. Koyrakh, L.; Luján, R.; Colón, J.; Karschin, C.; Kurachi, Y.; Karschin, A.; Wickman, K. *J. Neurosci.* **2005**, *25*, 11468.
15. Nurse, S.; Lacaille, J.-C. *Neuropharmacology* **1999**, *38*, 1733.
16. Quéva, C.; Bremner-Danielsen, M.; Edlund, A.; Ekstrand, A. J.; Elg, S.; Erickson, S.; Johansson, T.; Lehmann, A.; Mattson, J. P. *Br. J. Pharmacol.* **2003**, *140*, 315.
17. Froestl, W.; Mickel, S. J.; Hall, R. G.; von Sprecher, G.; Strub, D.; Baumann, P. A.; Brugger, F.; Gentsch, C.; Jaekel, J.; Olpe, H-R.; Rihs, G.; Vassout, A.; Waldmeier, P. C.; Bittiger, H. *J. Med. Chem.* **1995**, *38*, 3297.
18. Howson, W.; Mistry, J.; Btoekman, M.; Hills, J. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 515.
19. Alstermark, C.; Amin, K.; Dinn, S. R.; Elebring, T.; Fjellstrom, O.; Fitzpatrick, K.; Geiss, W. B.; Gottfries, J.; Guzzo, P. R.; Harding, J. P.; Holmen, A.; Kothare, M.; Lehmann, A.; Mattsson, J. P.; Nilsson, K.; Sunden, G.; Swanson, M.; von Unge, S.; Woo, A. M.; Wyle, M. J.; Zheng, X. *J. Med. Chem.* **2008**, *51*, 4315.