# Design, synthesis, and subtype selectivity of 3,6-disubstituted β-carbolines at Bz/GABA(A)ergic receptors. SAR and studies directed toward agents for treatment of alcohol abuse

Wenyuan Yin [a], Samarpan Majumder [b], Terry Clayton [a], Steven Petrou [c], Michael L. VanLinn [a], Ojas A. Namjoshi [a], Chunrong Ma [a], Brett A. Cromer [c,d], Bryan L. Roth [b], Donna M. Platt [e], James M. Cook [a,*]

[a] Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201-0413, United States
[b] Department of Pharmacology and Division of Medicinal Chemistry, University of North Carolina-Chapel Hill Medical School and School of Pharmacy and NIMH Psychoactive Drug Screening Program, 120 Mason Farm Road, 4072 Genetics Medicine Bldg, Chapel Hill, NC 27599-7365, United States
[c] Howard Florey Institute, University of Melbourne, Parkville, VIC 3010, Australia
[d] Department of Pharmacology, University of Melbourne, Parkville, VIC 3010, Australia
[e] Harvard Medical School, New England Primate Research Center, One Pine Hill Drive, PO Box 9102, Southborough, MA 01772-9102, United States

ABSTRACT

A series of 3,6-disubstituted β-carbolines was synthesized and evaluated for their in vitro affinities at $\alpha_x\beta_3\gamma_2$ GABA$_A$/benzodiazepine receptor subtypes by radioligand binding assays in search of $\alpha_1$ subtype selective ligands to treat alcohol abuse. Analogues of β-carboline-3-carboxylate-$t$-butyl ester (βCCt, 1) were synthesized via a CDI-mediated process and the related 6-substituted β-carboline-3-carboxylates 6 including WYS8 (7) were synthesized via a Sonogashira or Stille coupling processes from 6-iodo-βCCt (5). The bivalent ligands of βCCt (32 and 33) were also designed and prepared via a palladium-catalyzed homocoupling process to expand the structure–activity relationships (SAR) to larger ligands. Based on the pharmacophore/receptor model, a preliminary SAR study on 34 analogues illustrated that large substituents at position-6 of the β-carbolines were well tolerated. As expected, these groups are proposed to project into the extracellular domain (L$_{Di}$ region) of GABA$_A$/Bz receptors (see 32 and 33). Moreover, substituents located at position-3 of the β-carboline nucleus exhibited a conserved stereo interaction in lipophilic pocket L$_1$, while N(2) presumably underwent a hydrogen bonding interaction with H$_1$. Three novel β-carboline ligands (βCCt, 3PBC and WYS8), which preferentially bound to α1 BzR subtypes permitted a comparison of the pharmacological efficacies with a range of classical BzR antagonists (flumazenil, ZK93426) from several different structural groups and indicated these β-carbolines were 'near GABA neutral antagonists'. Based on the SAR, the most potent (in vitro) $\alpha_1$ selective ligand was the 6-substituted acetylenyl βCCt (WYS8, 7). Earlier both βCCt and 3PBC had been shown to reduce alcohol self-administration in alcohol preferring (P) and high alcohol drinking (HAD) rats but had little or no effect on sucrose self-administration.[1–3] Moreover, these two β-carbolines were orally active, and in addition, were anxiolytic in P rats but were only weakly anxiolytic in rodents. These data prompted the synthesis of the β-carbolines presented here.

Published by Elsevier Ltd.

## 1. Introduction

### 1.1. Alcoholism

Alcohol addiction and dependence remain significant public health concerns, impacting physical and mental well-being, family structure and occupational stability.[4] While advances have been made in the development of novel therapies to treat alcohol-ism,[5–8] alcohol-dependent individuals represent a heterogeneous group,[9–11] and it is unlikely that a single pharmacological treatment will be effective for all alcoholics. Hence, a better understanding of the neuromechanisms which regulate alcohol seeking behaviors and the design of clinically safe and effective drugs that reduce alcohol addiction and dependence remain a high priority.[7,12] While the precise neuromechanisms regulating alcohol-seeking behaviors remain unknown, there is now compelling evidence that the GABA$_A$ receptors within the striatopallidal and extended amygdala system are involved in the 'acute' reinforcing actions of alcohol.[13–18] Among the potential GABA$_A$ receptor isoforms within the VP regulating alcohol-seeking behaviors, GABA

* Corresponding author. Tel.: +1 414 229 5856; fax: +1 414 229 5530.
E-mail address: capncook@uwm.edu (J.M. Cook).

receptors containing the α1 receptor subtype (GABA α1) appear preeminent. Thus, Criswell et al. observed that acute alcohol administration selectively enhanced the effects of iontophoretically applied GABA in the ventral pallidum (VP).[19,20] However, no effects were seen in the septum, ventral tegmental area (VTA), and CA1 hippocampus. These data suggest the α1 Bz/Gaba(A)ergic receptor plays an important role in alcohol-motivated behaviors. Research on the neuroanatomical basis of alcohol reward has shown that the NACC, VTA, VP, central amygdala (CeA), and hippocampus are all involved in GABAergic regulation of ethanol (EtOH) reinforcement.[2,21–24] Other investigators have identified a dense reciprocal projection from the VP to the NACC,[25–27] and many of these have been found to be GABAergic neurons.[28–30] The NACC is now well established as a substrate that regulates the reinforcing properties of abused drugs.[13] Finally, immunohistochemical[31,32] and in situ hybridization studies[33–35] have demonstrated that the VP contains one of the highest concentrations of mRNA encoding the α1 subunit in the CNS. These findings, together with pharmacological studies suggesting the VP plays a role in reward-mediated behaviors of psychostimulants and opiates,[28,36–39] suggest a possible role of the VP-α1 receptors in the euphoric properties of alcohol. Findings of previous studies concluded that inhibition of VP-α1 receptors by the α1-preferring antagonist 3-PBC produced marked reductions on alcohol-maintained responding.[9,15,40,41] The α1-mediated suppression at the VP level by 3-PBC showed a high degree of neuroanatomical specificity. Specifically, the α1-mediated suppression was not observed with the more dorsal placements in the NACC or caudate putamen. The failure of 3-PBC to alter alcohol self-administration in the NACC/striatum is in agreement with previous research which has consistently reported a lack of expression of the α1 transcript in the NACC and caudate putamen.[31–35]

An understanding of the neuromechanisms that regulate alcohol drinking is key in the development of drugs to treat alcohol addiction and dependence in humans.[2] In recent years, much evidence has accumulated in favor of the GABA system;[22,23,42,43] however much remains unknown about the role of specific GABA_A receptor subtypes in regulating ethanol reinforcement. This is due to both a lack of high affinity and selective ligands capable of discriminating among the GABA_A receptor subunits and the heterogeneity of various subunits within the known alcohol reward circuitry.[31,34] Of the potential GABA_A receptors involved in the reinforcing properties of alcohol, evidence suggests the α1 subtype within the VP may play an important role in regulating alcohol-seeking behaviors, as mentioned above. The VP contains one of the highest distributions of α1 subunits in the mesolimbic system.[32–35] Finally, acute ethanol administration has been reported to selectively enhance the effects of iontophoretically applied GABA in the VP. These effects correlate highly with [$^3$H] zolpidem binding (an α1 subtype selective agonist).[19,20]

The GABA_A receptor is the major inhibitory neurotransmitter receptor of the central nervous system (CNS) and the site of action of a variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, neuroactive steroids, anesthetics, and convulsants.[44] It is now clear that these receptors regulate the excitability of the brain, anxiety, muscle tone, circadian rhythms, sleep, vigilance, memory, and learning.[44] There are several disease states thought to be associated with the improper functioning of this system, including anxiety, epilepsy,[45] insomnia,[46] depression and bipolar disorder,[47,48] schizophrenia,[49] as well as mild cognitive impairment and Alzheimer's disease.[50] A role of GABA_A receptors in drug and alcohol abuse has also been reported.[51–53] Briefly, GABA_A receptors are composed of five subunits that form a central chloride channel and can belong to different subunit classes. A total of 19 subunits (6α, 3β, 3γ, 1δ, 1ε, 1π, 1θ, 3ρ) of the GABA_A receptor have been cloned and sequenced from

the mammalian nervous system.[54–59] All these polypeptides possess an approximate molecular mass of ∼50 kD and are structurally related.

To evaluate the role of the α1 receptor in regulating alcohol reinforcement, 3-propoxy-β-carboline hydrochloride (3-PBC), a mixed benzodiazepine (BDZ) agonist–antagonist with binding selectivity at the α1 receptor was developed.[2] Compared with the prototypical BDZ agonist zolpidem, 3-PBC exhibited a slightly higher binding selectivity for the α1 receptor.[60,61] Preliminary behavioral studies in several species (e.g., rats, mice, and primates) show that 3-PBC is a BDZ antagonist, exhibiting competitive binding-site interactions with BDZ agonists at low to moderate doses (2.5–15 mg/kg).[60,62,63] At higher doses (15–60 mg/kg), 3-PBC produces anxiolytic effects in the plus maze that are comparable with those of chlordiazepoxide in alcohol preferring (P) rats.[62–65] Thus given the proposed subunit composition of the GABA receptors within the CeA,[31,32,34,66] pharmacological compounds capable of exploiting the α1, α2, and α3 subunit-containing GABA_A receptors represent optimal tools to evaluate the role of GABA_A receptors in alcohol reinforcement and better understand neurobehavior and ethanol responding.

### 1.2. α1 Subtype selective ligands

The β-carboline-3-carboxylate-*t*-butyl ester (βCCt) is a mixed benzodiazepine agonist–antagonist ligand with binding selectivity at α1 receptors;[2,62,67] βCCt also exhibits some affinity (albeit lower) for both α2 and α3 receptors. Behavioral studies in several species (e.g., rats, mice, primates) show that βCCt is a BDZ antagonist exhibiting competitive binding-site interactions with BDZ agonists over a broad range of doses.[60,62,68–72] Other studies show that βCCt produces anxiolytic effects in rodents[62] and potentiates the anti-conflict response induced by α1 subtype agonists in primates.[70] Thus, βCCt displays a weak agonist or antagonist profile depending on the behavioral task, species, and dose employed.

In studies involving the α1 subtype, βCCt, and 3-PBC were observed to selectively reduce alcohol-motivated behaviors in a variety of experiments.[2,73] However, unlike the α5 selective inverse agonist RY-23, both the β-carboline antagonists βCCt and 3-PBC displayed mixed weak agonist–antagonist profiles in vivo in alcohol P and HAD rats. Therefore, in addition to being able to study the molecular basis of alcohol reinforcement, α1 Bz β-carboline ligands which display mixed agonist–antagonist pharmacology in alcohol P and HAD rats may be capable of reducing alcohol intake while eliminating or greatly reducing the anxiety associated with habitual alcohol, abstinence or detoxification. Thus, these types of ligands may be ideal clinical agents for the treatment of alcohol dependent individuals.[2,73]

Consequently, several series of structurally different compounds have been synthesized which possess some α1 subtype selectivity.[67,74–77] The discovery of high affinity, saturable, and stereospecific ligands for the BzR has been coupled with the demonstration that β-carbolines exhibited an affinity for the BzR.[78–85] Some of these agents act on the BzR to induce effects that are functionally opposite (inverse agonists/antagonists) to those of classical BDZs. Consequently, the affinities of a wide variety of β-carbolines have been reported on synaptosomal membranes from this laboratory,[60,72,78,80,81,86–90] and the laboratories of others,[91–96] and this prompted the study of the binding affinities of a series of β-carbolines[67] at five recombinant GABA_A/BzR subtypes (α1β3γ2, α2β3γ2, α3β3γ2, α5β3γ2 and α6β3γ2) expressed from recombinant human cell lines.[91,97] In general, this series of β-carboline ligands exhibited some selectivity at α1 receptor subtypes including βCCt (**1**) and 3-PBC (**2**).[2,3] These two ligands displayed a 20-fold and 10-fold selectivity, respectively, for the α1 subtype over the α2 and α3 receptors, as well as over 150-fold selectivity

**1** βCCt

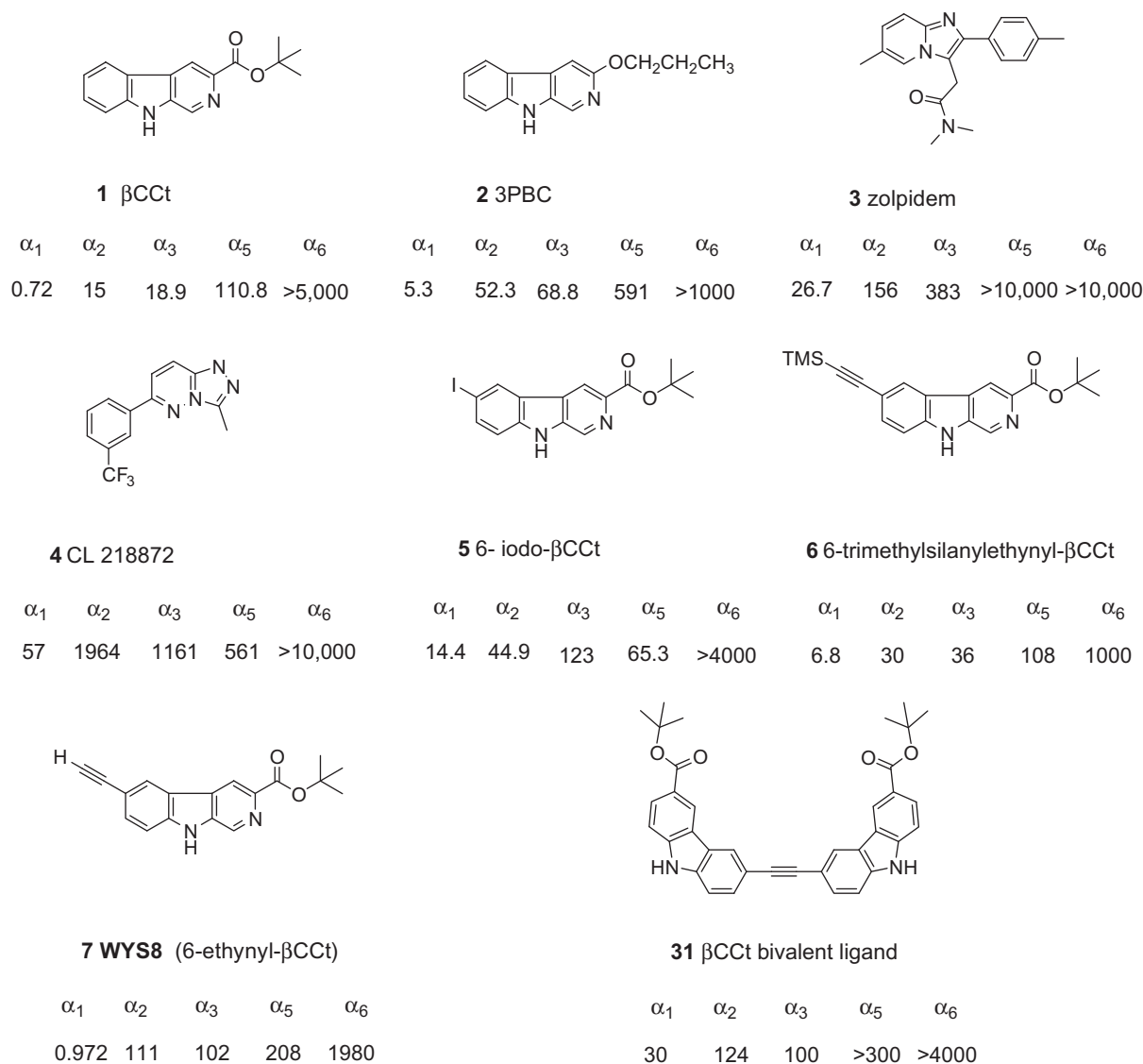| $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|
| 0.72 | 15 | 18.9 | 110.8 | >5,000 |

**2** 3PBC

| $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|
| 5.3 | 52.3 | 68.8 | 591 | >1000 |

**3** zolpidem

| $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|
| 26.7 | 156 | 383 | >10,000 | >10,000 |

**4** CL 218872

| $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|
| 57 | 1964 | 1161 | 561 | >10,000 |

**5** 6- iodo-βCCt

| $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|
| 14.4 | 44.9 | 123 | 65.3 | >4000 |

**6** 6-trimethylsilanylethynyl-βCCt

| $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|
| 6.8 | 30 | 36 | 108 | 1000 |

**7 WYS8** (6-ethynyl-βCCt)

| $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|
| 0.972 | 111 | 102 | 208 | 1980 |

**31** βCCt bivalent ligand

| $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|
| 30 | 124 | 100 | >300 | >4000 |

**Figure 1.** In vitro binding affinities of a series of $\alpha_1$ selective ligands ($K_i$ in nM).

for the $\alpha_1$ site over the $\alpha_5$ subtype.[2,3] βCCt (**1**) was more selective at the $\alpha_1$ subtype in vitro than the classical $\alpha_1$ selective agonists zolpidem (**3**) and CL 218872 (**4**) (Fig. 1).[60,98,99] A number of in vitro and in vivo studies employing $\alpha_1$ (e.g., zolpidem, CL 218872,[68] βCCt, and 3-PBC[3]) selective ligands suggest the $\alpha_1$-containing GABA$_A$/Bz receptors of the ventral pallidum (VP) play an important role in regulating alcohol's neurobehavioral effects; particularly alcohol's reinforcing properties as mentioned above.[2,3,19,20,35]

## 2. Structure–activity relationships

A predictive 3-D QSAR pharmacophore/receptor model for inverse agonist/antagonist β-carbolines was initially developed via Comparative Molecular Field Analysis (CoMFA) and later refined.[100,101] Affinities of ligands from 15 different structural classes have been evaluated.[61] Based on this CoMFA study of a series of β-carbolines, Huang et al. reported that β-carbolines bind to all diazepam sensitive (DS) sites of the BzR with some selectivity at the $\alpha_1$-containing receptor isoform and this was confirmed by the in vitro binding affinity of these ligands.[102] A lipophilic region ($L_{Di}$) of the pharmacophore receptor model appears to be larger in the $\alpha_1$, $\alpha_2$ and $\alpha_3$-containing receptor isoforms and important for $\alpha_1$ subtype selectivity.[61] More recently, during the design and

synthesis of βCCt-related bivalent ligands,[103–106] it was found that a series of 3,6-disubstituted β-carbolines (see Fig. 1), including 6-iodo-βCCt (**5**) and 6-trimethylsilanyl-ethynyl-βCCt (**6**) (Fig. 1) possessed $\alpha_1$ subtype selectivity.[103]

The rigidly linked linear bivalent ligands of βCCt at position '6' did bind to BzR receptors with some $\alpha_1$ subtype selectivity and may provide the desired $\alpha_1$ selectivity through specific occupation of the $L_{Di}$ region of the pharmacophore/receptor model.[103] Although the two 3,6-disubstituted-β-carbolines **5** and **6** are less potent than βCCt (**1**), the potent binding affinities observed for **5** and **6** at the $\alpha_1$ subtype has stimulated the synthesis of the βCCt analogs: 3-substituted-β-carbolines as well as 3,6-disubstituted-β-carbolines.

On the other hand, these studies also indicated that the selectivity of GABA$_A$/BzR site ligands could be described in relation to binding and pharmacological efficacy in vitro. This efficacy was based on the capacity of a ligand to modulate GABAergic function.[54] BzR ligands act to modulate chloride flux over a continuum ranging from positive to negative modulation, with neutral antagonists acting theoretically, at a point on the continuum, with zero intrinsic efficacy (e.g., they bind to the receptor but exhibit no activity).[74] Consequently, the pharmacological profiles of βCCt and 3-PBC at recombinant $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_4\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$

and $\alpha_6\beta_3\gamma_2$ receptor subtypes expressed in *Xenopus* oocytes were investigated.[2,3,64]

The results of this study illustrated that βCCt was a near 'neutral' antagonist (i.e., little or no efficacy) at all receptor subtypes. In fact, the level of intrinsic efficacy of βCCt in oocytes was less at some receptor subtypes than the classical non-selective antagonist flumazenil (Ro 15-1788, for which intrinsic efficacy at all BZ-sensitive GABA$_A$ subtypes was relatively low, but not zero). To date, no compound has been characterized that exhibits zero efficacy at all BzR subtypes, raising the possibility that a compound labeled as an 'antagonist' may indeed exhibit functional activity given the right circumstances. For example, more recently, the efficacies of both βCCt and 3-PBC in the selective reduction of alcohol responding and production of anxiolytic effects were demonstrated in P and HAD rats following oral administration.[107] When compared with naltrexone treatment, these reductions in alcohol responding were more selective and longer in duration.[107] In summary, the antagonist βCCt exhibited either a neutral or low-efficacy agonist response at GABA receptors in oocytes. Although there has been some debate in the literature at present as to whether a ligand's binding or efficacy selectivity was 'the more salient factor' in determining a ligand's capacity to function as an alcohol antagonist,[3,67,107] the knowledge of the efficacy of an individual putative anti-alcohol reward ligand across all GABA$_A$ receptors is indeed critical to the knowledge of their mode of action in the CNS.

Based on the limited availability of data on the series of $\alpha_1$ 'binding' and 'efficacy' selective β-carbolines (βCCt, 3PBC) as anti-alcohol agents,[108] the present study was designed to expand the SAR and search for better $\alpha_1$ subtype selective ligands. These compounds may be promising modulators of alcohol-related co-morbid behaviors in alcohol dependence via the GABA$_A$/BzR system. Although recent evidence suggests a salient role for GABAergic mechanisms in the regulation of excessive alcohol drinking and the negative affective states associated with abstinence, decreased GABAergic tone stemming from chronic alcohol use and withdrawal may serve to generate anxiety.[109] Thus, compounds that enhance GABAergic tone may be effective and safe treatments for both excessive alcohol drinking and the negative affective states associated with abstinence and may represent novel pharmacotherapies to treat alcoholism.

In this regard, the chemistry and pharmacological evaluation of a series of structurally modified analogues of βCCt (**1**) as selective and potent $\alpha_1$ subtype-preferring ligands are described. The synthesis of the α1 selective compound **7** (WYS8) and the structure–activity relationships (SAR) of 3,6-disubstituted β-carbolines are also presented. The established pharmacophore/receptor model[61,110] of BDZ binding sites was employed to design ligands with respect to the L$_{Di}$ region at position-6, as well as characterize the binding pocket L$_1$ at position-3. Protein-ligand docking of the α1 subtype GABA$_A$ receptor protein and WYS8 illustrated the agreement between the pharmacophore/receptor model and BzR site prediction based on homology modeling.[110–113]

## 3. Chemistry

The synthesis of the ligands under study is outlined in Schemes 1–3. The important precursor β-carboline-3-carboxylate ethyl ester (βCCE, **8**) and its corresponding acid (**9**) were the intermediates required for large-scale synthesis of βCCt (**1**), as well as an intermediate required for the synthesis of the new β-carbolines. As outlined in Scheme 1, D,L-tryptophan **10** was converted into tetrahydro-β-carboline (**11**) via a Pictet–Spenger reaction on kilogram scale. Fischer esterification of **11**, followed by oxidation with activated MnO$_2$ provided the intermediate BCCE (**8**) on 200 g scale.

Hydrolysis of ester **8** to provide the acid **9**, was then followed by esterification in *t*-butanol with CDI to provide BCCt (**1**). The synthesis of 3PBC·HCl (**2**) was more difficult to scale up due to the complex last step (**14**→**2**). It began with βCCE (**8**) from Scheme 1, which was heated with hydrazine to furnish hydrazide (**13**) in 82% yield. The hydrazide (**13**) was stirred with nitrous acid to provide an azide, which was unstable, and was converted into 3-amino-β-carboline (**14**) when stirred with acetic acid (Scheme 1) via a Curtius rearrangement. The last step, originally developed on a 100 mg scale, has now been scaled up to 4 g levels to furnish 3PBC hydrochloride salt in reasonable yield for studies in primates.

In Scheme 2, the β-carboline alkyl esters **16**–**20** as well as chiral βCCt analogs **21**–**24**, and **25**, **26** were prepared via the CDI-mediated process described above (see Scheme 1).[113] Briefly, when β-carboline-3-carboxylic acid **9** was treated with 1,1-carbonydiimidazole (CDI) in dry DMF, the imidazole derivative **27** which resulted was subsequently transformed into the desired esters by treating it with the corresponding alcohols (individually) in the presence of DBU in a one-pot sequence. The key potential $\alpha_1$ chiral selective analogs CMD-30 *R/S* isomers (**21** and **22**) can be synthesized by the CDI method in 90% yield (individually) on 10 g scale. The required starting chiral alcohols were obtained by asymmetric reduction of the corresponding trifluoromethyl ketones with (+)-DIP-chloride,[114,115] or the (−)-DIP-enantiomer. The 6-substituted-iodo-β-carboline-3-carboxylates **28** were then prepared as intermediates to generate different functionality at position-6 through a palladium-mediated cross coupling process. For example, as illustrated in Scheme 2, βCCt (**1**) was treated with I$_2$/CF$_3$COOAg in chloroform to provide 6-iodo-βCCt **5** (see also **28a**) in 80% yield and the 6-substituted targets **29**–**31** were obtained in 65–83% yields via a Stille coupling process employing commercially available substituted tributyl-stannanes. The substitution by halogen occurred at position-6 as indicated by analysis of **5** (or **28a**) by NMR spectroscopy especially with One Dimensional Nuclear Overhauser Effect (NOE) experiments.

Depicted in Scheme 3 are the synthetic routes for the βCCt-related bivalent ligands **32** and **33**.[103] In order to efficiently effect a palladium-mediated Sonogashira process at position-6 of β-carbolines (a reactive electron-rich indole heterocycle), protection/deprotection of the indole N$_a$–H group in β-carbolines **34** and **35** was necessary. The Boc protected 6-ethynyl-β-carboline-3-carboxylic acid *t*-butyl ester (**34**) was prepared directly from 6-substituted acetylenyl βCCt (**7**), which was initially termed **WYS8**. The common intermediate, iodo-βCCt **5** (see also **28a**) was then converted into the 6-substituted trimethylsilylacetylenyl βCCt (**6**) via a Sonogashira coupling process.[116,117] At this point, TBAF was employed to remove the trimethylsilyl group to provide the 6-substituted acetylenyl βCCt analog **WYS8** (**7**) as well.

The ester **7**, was then protected with a Boc group at the N(1) position to afford **34** under standard conditions. A Sonogashira process was then employed to couple **34** with Boc protected iodo-βCCt (**35**) to provide the rigid two-carbon linked bivalent ligand **32** of βCCt. The Boc protecting group was removed thermally by heating in cumene at high dilution and bivalent ligand **32** was obtained. The bisacetylenic bridged ligand **33** was synthesized from the Boc protected 6-ethynyl-βCCt **34** via a homocoupling process,[118] followed by the removal of the Boc group under thermal conditions in cumene at high dilution.

## 4. Results and discussion

### 4.1. βCCt bivalent ligands

The in vitro biological protocols employed in the present study follow the published procedure[119,120] and are detailed in Section 6.
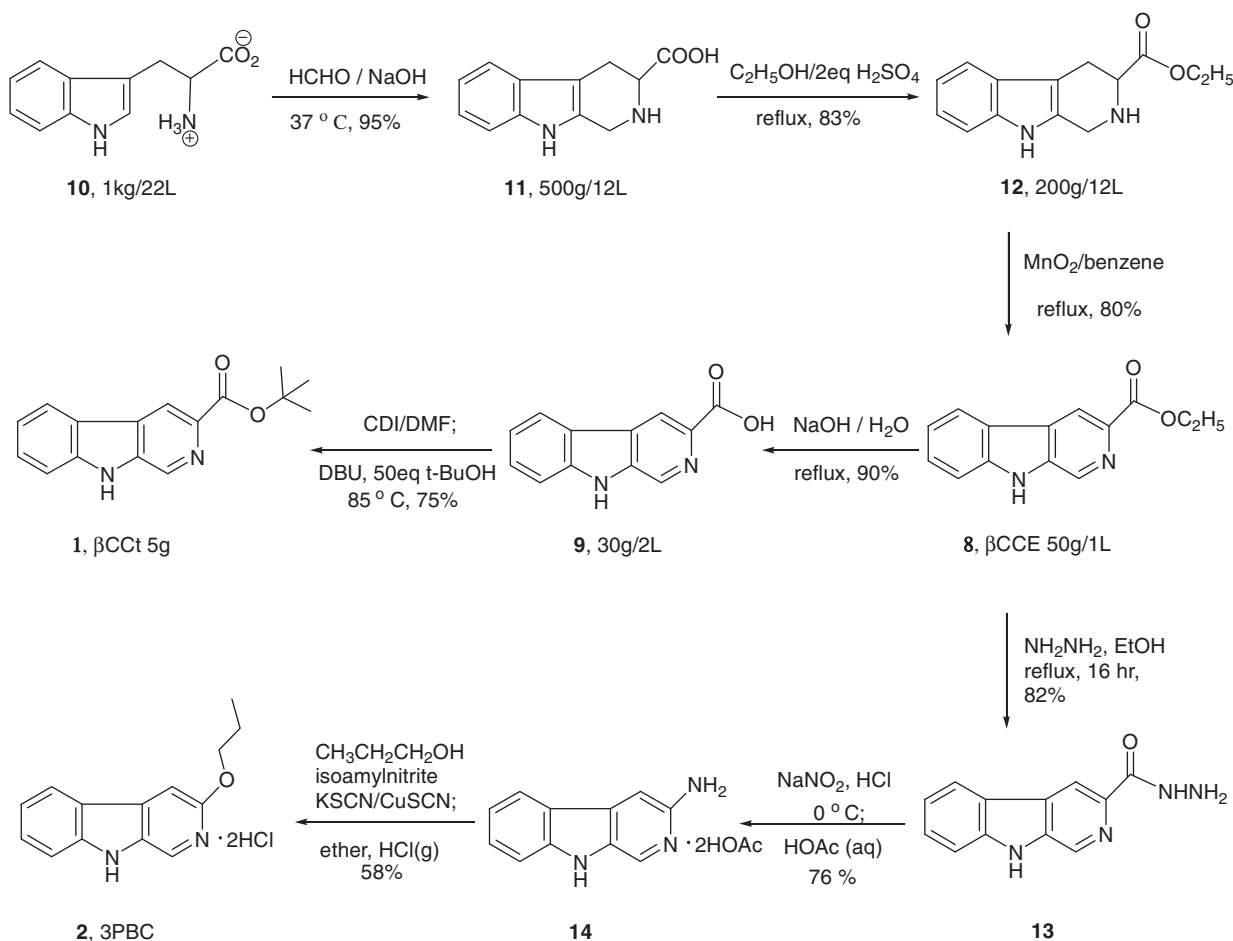
**Scheme 1.** Large scale synthesis of βCCt and synthesis of 3PBC.

Although the $\alpha_1\beta_3\gamma_2$ BzR/GABAergic subtype is very similar in structure to the $\alpha_2$ and $\alpha_3$ subtypes, there are slight differences.[61,121] One major difference is in region $L_{Di}$, which appears larger in the $\alpha_1$ subtype than in either the $\alpha_2$, or $\alpha_3$ or $\alpha_5$ subtypes. This is located near position-6 of βCCt (1) (Fig. 3).[60,110,122]

The western region of the pharmacophore/receptor model tolerates positive lipophilic interactions in this area that corresponds to region $L_{Di}$ (a region in the pharmacophore adjacent to the extracellular domain of the receptor) of the unified pharmacophore/receptor model. In this region, bulky substituents are tolerated and occupation of this area with substituents appears to enhance affinity at $\alpha_1$ subtypes. This knowledge provided an opportunity to introduce a linker between two pharmacophoric β-carboline-3-carboxylate residues in order to design selective and rigid bivalent ligands. As described in the Introduction, initial efforts to find a novel series of $\alpha_1$-preferring ligands focused on design and synthesis of βCCt bivalent ligands. Although the $\alpha_1$ subtype selectivity was not amplified with the particular acetylenyl linked bivalent ligand 32, the ligand does bind preferentially at $\alpha_1$ subtypes (Table 1). It was proposed the two-carbon linker was not long enough and that crowding between the second βCCt unit and the receptor protein decreased the binding affinity at the $\alpha_1$ subtype, thereby negating some of the potential selectivity. However, these rigidly linked linear bivalent ligands 32 and 33 fit the GABA$_A$/BzR pharmacophore/receptor model very well (Fig. 4).[110] The unit at C-6, presumably, protrudes into the extracellular domain of the BzR, as previously expected,[102,103] and bound to BzR with some $\alpha_1$ subtype selectivity.[102,103] To our

knowledge these are the first two bivalent ligands in the β-carboline series, which bind to BzR. Further pharmacological evaluation in vivo of the βCCt bivalent ligand with the longer rigid linker should shed light on the above hypothesis and this would also provide some tools to determine the size and exact location of the $L_{Di}$ region.

### 4.2. WYS8

A series of 6-substituted-β-carboline-3-carboxylates have been synthesized and bound in vitro to the $\alpha_1\beta_3\gamma_2$ BzR subtype preferentially as compared to other subtypes (see Tables 1–3).[61,102] These ligands have also been modeled in the GABA$_A$/BzR pharmacophore model, and the 6-substitutents align well in the $L_{Di}$ region.[123] Occupation of this region should lead to enhanced selectivity of a ligand at the $\alpha_1$-containing isoform. Among the new 3,6-disubstituted-β-carbolines, 6-trimethylsilanylethnyl-βCCt 6 has been recently synthesized and found in vitro to prefer the $\alpha_1$ subtype. However, the most selective ligand for the $\alpha_1$ subtype was WYS8 (7). This $\alpha_1$ subtype selective ligand was 100-fold more selective over the other subtypes. This was the most $\alpha_1$ subtype selective ligand reported, to date, to these authors' knowledge. This 6-substituted acetyleny βCCt 7 was 214-fold more selective for $\alpha_1$ isoforms over $\alpha_5$ isoforms. Studies of SAR in Table 1 confirmed the occupation of region $L_{Di}$ of the receptor pharmacophore model did enhance $\alpha_1$ selectivity in comparison to the affinity of the non-selective ligand diazepam or the $\alpha_5$ selective ligand, RY080. As illustrated in the two dimensional Figure 5, full occupation of the $L_{Di}$ lipophilic region
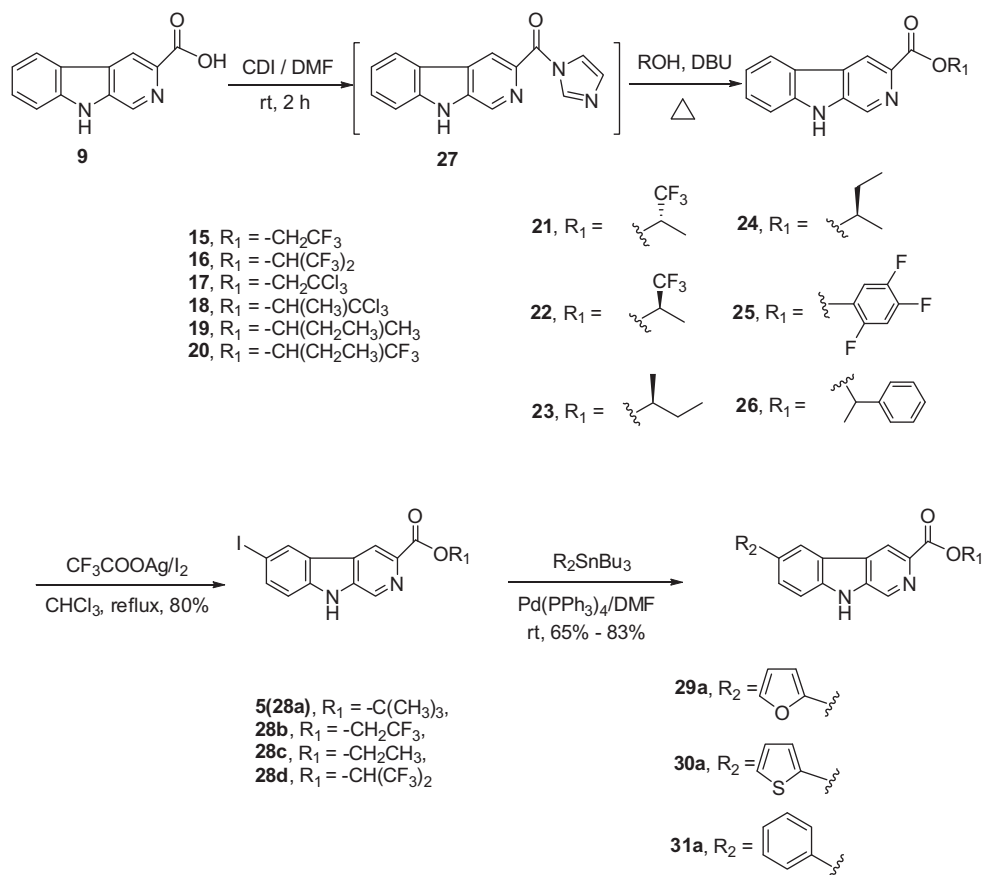
**Scheme 2.** CDI-mediated esterification of 3-substituted β-carbolines followed by the conversion into 3,6-disubstituted β-carbolines.

by β-carbolines may account for the potency/selectivity of this class of ligands at the $\alpha_1$ subunit. Analysis of the in vitro binding data for this series of bulky 6-substituted β-carbolines (Table 1) has shown some selectivity for the $\alpha_1$ receptor subtype. In addition, it is important to note that binding affinity in this series of ligands of greater than 400 nM usually results in zero efficacy at the subtype at pharmacologically relevant concentrations.

Homology models employed here of the GABA$_A$ receptor were as described previously[111,124] except that a number of alternative models were considered for loop C, which was two residues shorter than the template and hence built from a loop database. The final model was selected based on assessment of model quality[125] and consistency with published mutational data,[126–130] particularly with the T207 side-chain appropriately positioned facing the benzodiazepine-binding pocket. Positioning of WYS8 in the BzR was executed using a genetic algorithm (FlexiDock®). Flexible docking provides a means of docking ligands into protein active sites.

### 4.3. 3-Substituted β-carbolines

It was initially believed by Braestrup, Loew, and others that an ester moiety at position-3 of β-carbolines was required for a ligand to exhibit high affinity binding at Bz binding sites.[84,95,131,132] However, high affinity binding of β-carbolines including the antagonist 3-propoxy β-carboline (3-PβC, Table 2b) demonstrated this was not the case.[81,88,89] Examination of data from additional studies[78,88,89] have suggested that at least two factors affected high affinity binding at BzR with respect to 3-alkoxy substituted β-carbolines,[60,79,102,123] one of which was the lipophilicity of the substitu-

ent which interacted at L$_1$. The L1 pocket tolerates linear groups up to four carbons in length. Comparison of the in vitro receptor binding affinity of the ligands depicted in Table 2b indicated the ethers **36** and **37** bind potently to $\alpha_1$ subtypes while **34** does not; binding affinity is lost, illustrating that the substituent at the 3-position is to large to allow the ligand to bind. Likewise, the 3-benzyloxy β-carboline **35** is also too bulky to fit the L1 pocket despite its lipophilic nature. The second factor was the ability of the substituent at position-3 to release electron density to the pyridine ring. This enhanced the basicity of the nitrogen atom at N(2) which resulted in a greater ligand-receptor interaction at H$_1$. Analysis of the binding affinities of the novel trifluoroalkyl esters of β-carboline-3-carboxylic acid further supported this hypothesis (Table 2a). The trifluoroalkyl esters exhibited reduced binding affinity at all receptor subtypes when compared to their corresponding alkyl esters (**20** vs **19**, **15** vs βCCE). Since the trifluoromethyl was a strong electron-withdrawing group, when compared to the corresponding alkoxycarbonyl moiety, the 3-trifluoroalkoxycarbonyl substituent would decrease electron density to the pyridine (N2) ring reducing the basicity of the nitrogen atom. This would result in a weaker ligand-receptor interaction at H$_1$. In addition, the trifluoroalkyl group was less lipophilic than the corresponding alkyl moiety, which may result in a weaker interaction at L$_1$. Ramachandran and Hanzawa have reported that trifluoromethyl groups are nearly as large as isopropyl or t-butyl functions.[114,133] It was possible, the trifluoromethyl substituted ligands are simply too large to exert high affinity binding; however, βCCT (**1**), WY-B-24 (**25**) and CM-A-77 (**26**) all bound with good potency to α1 BzR subtypes (see Tables 1 and 2), and these ester functions occupy a large molecular volume.
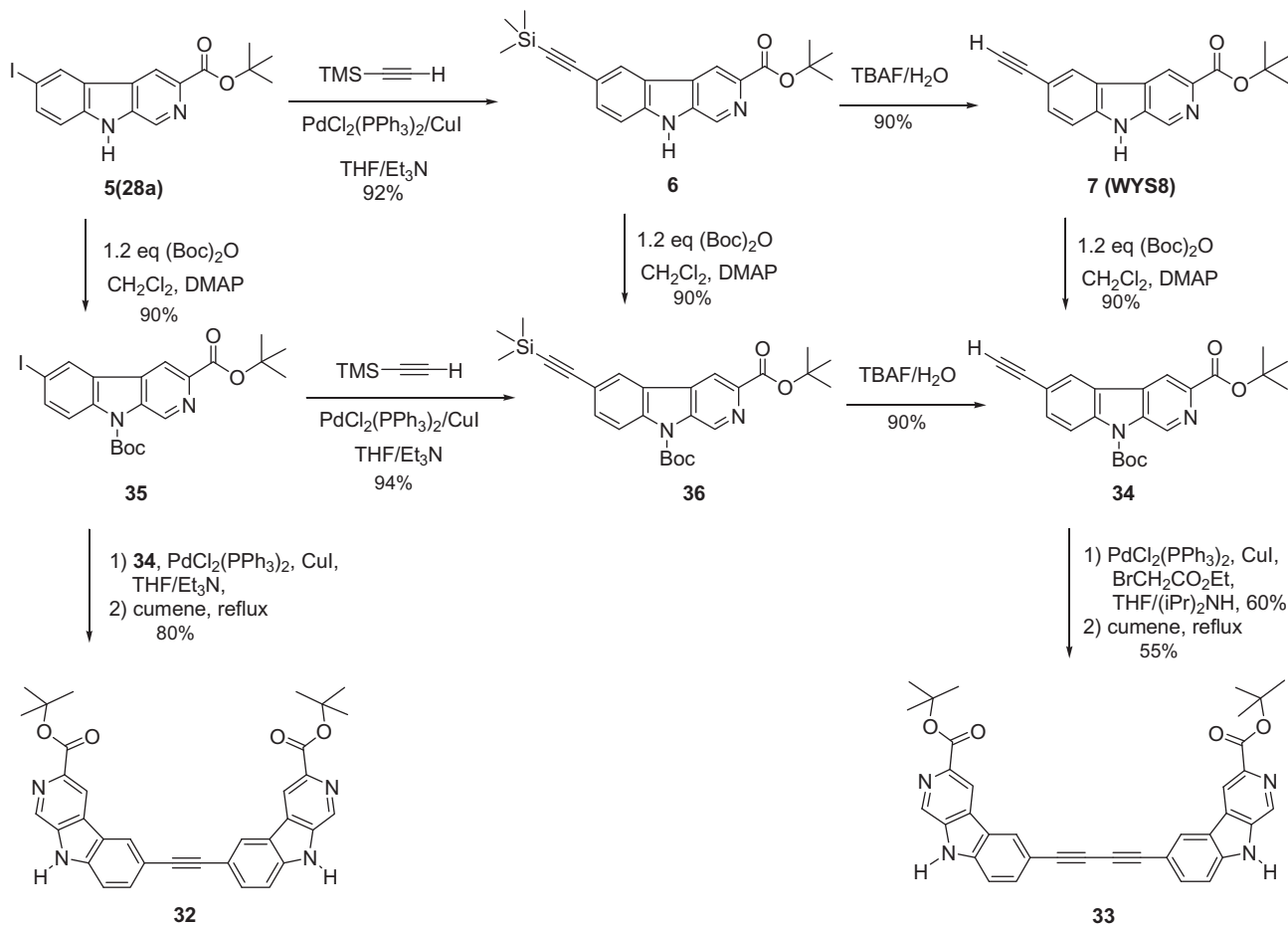
**Scheme 3.** Preparation of 6-substituted acetylenyl βCCt (**7**, WYS8) and related bivalent ligands.
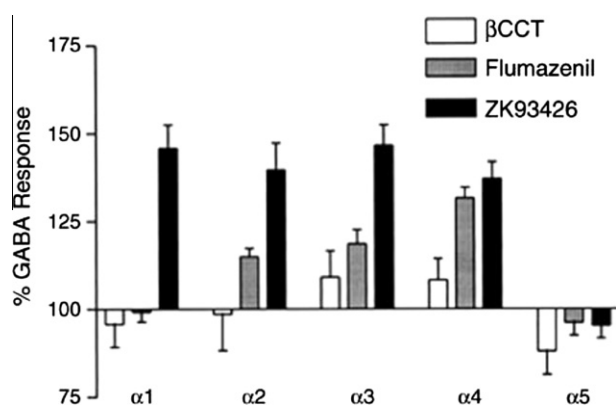


**Figure 2.** Efficacy of βCCt in modulating GABA at recombinant GABA$_{1-5}$ receptors[2] in *Xenopus* oocytes: comparison with other BzR antagonists. Modulation of GABA$_A$ $\alpha 1\beta 3\gamma 2$, $\alpha 2\beta 3\gamma 2$, $\alpha 3\beta 3\gamma 2$, $\alpha 4\beta 3\gamma 2$, and $\alpha 5\beta 3\gamma 2$ receptor subunit combinations expressed in *Xenopus* oocytes by βCCt (open bars), flumazenil (shaded bars), and ZK 93426 (black bars). A saturating concentration (1–10 μM) was coapplied over voltage-clamped oocytes along with an EC$_{50}$ of GABA.

## 4.4. Chiral 3-substituted β-carbolines

Examination of the binding data for the enantiomeric pair of β-carboline sec-butyl esters **23** and **24** (Table 3) indicated that the (*R*)-enantiomer **24** bound tighter to the receptor subtypes than the (*S*) isomer **23**. Although both enantiomers exhibited approximately a fourfold selectivity for the $\alpha 1\beta 3\gamma 2$ subtype, the (*R*) isomer

remained more potent in vitro at all 5 BzR subtypes. Because the receptor subtype selectivity remained about the same for the (*R*) and (*S*) isomers, this indicated the stereoenvironment in lipophilic pocket L$_1$ was highly conserved across the entire series of BzR subtypes in agreement with earlier work on the binding affinities of the enantiomers of the framework-constrained 4,5-substituted pyrroloimidazobenzodiazepines.[134] It is possible that lipophilic pocket L$_1$ is simply a large area in the pharmacophore/receptor model with only small steric differences between receptor subtypes. More work will be required to determine if this is the case. A similar result was observed in the case of (*R*) and (*S*) isomers of CMD-30. The (*R*)-enantiomer CMD-30 *R* (**22**) bound slightly tighter to the receptor subtype than the (*S*) isomer (**21**) with almost 70-fold more selectivity for the $\alpha_1$ subtype over the $\alpha_5$ isoform. In addition, previously it was reported that a hydrogen bond between the N(9) H atom of a β-carboline and the secondary site A$_2$ in the receptor pharmacophore was required for potent inverse agonist activity in vivo.[78,79] Therefore, a series of ligands with the Boc protection at position-9 such as **40** and **41** were evaluated and were not $\alpha_1$ subtype selective ligands. In fact, they did not bind to BzR at all in agreement with previous work.[123]

## 4.5. Efficacy of $\alpha_1$-preferring ligands in oocytes at GABA$_A$ receptor channels

The physiological efficacy of βCCt, as compared to other Bz antagonists, was investigated across all diazepam sensitive (DS) receptor subunits at recombinant $\alpha_1$, $\alpha_2$, $\alpha_3$, and $\alpha_5$ receptor subunits in the *Xenopus* oocytes assay and is depicted in Figure 2 by
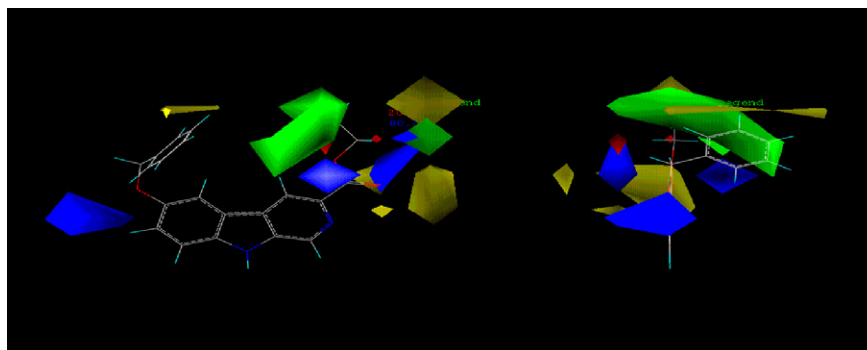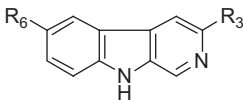
**Figure 3.** Orthogonal views of CoMFA contour maps for the affinity of 6-benzyl-substituted β-carbolines at the $\alpha_1\beta_3\gamma_2$ BzR. Orthogonal view of CoMFA contour maps for the $\alpha_1\beta_3\gamma_2$ receptor subtype with 6-benzyl-substituted β-carbolines modeled by Huang.[60] Green contours represent areas of positive steric interaction at a contribution level of 85%, which would result in reduced binding affinity. Blue contours represent areas of positive charge interaction at a level of 85%, which would increase the affinity of a ligand.

**Table 1**
Affinities ($K_i$ = nM) of 3,6-disubstituted β-carbolines at $\alpha_x\beta_3\gamma_2$ ($x$ = 1–3, 5, 6) receptor subtypes

| Ligands | R6 | R3 | $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_4$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|---|---|---|---|
| **1**(βCCt) | **H** | **$CO_2tBu$** | **0.72** | **15** | **18.9** | **1000** | **111** | **>5000** |
| **8**(βCCE) | **H** | **$CO_2Et$** | **1.2** | **4.9** | **5.7** | **ND** | **26.8** | **2700** |
| **2**(3-PBC) | H | OnPr | 5.3 | 52.3 | 68.8 | 1000 | 591 | >1000 |
| **6**(WYB14) | TMS≡ | $CO_2tBu$ | 6.8 | 30 | 36 | 2000 | 108 | 1000 |
| **6b**(WY-B-25) | TMS≡ | $CO_2CH_2CF_3$ | 17 | 59 | 88 | 200 | 1444 | >3000 |
| **6c**(WY-B-99-I) | TMS≡ | $CO_2Et$ | 4.4 | 4.5 | 5.58 | 2000 | 47 | 2000 |
| **7**(WYS8) | **H≡** | **$CO_2tBu$** | **0.972** | **111** | **102** | **2000** | **208** | **1980** |
| **7b**(WY-B-26-2) | H≡ | $CO_2CH_2CF_3$ | 4.5 | 44.6 | 42.7 | 2000 | 124 | 2000 |
| **28a**(iodo-βCCt) | I | $CO_2tBu$ | 14.4 | 44.9 | 123 | >4000 | 65.3 | >4000 |
| **28b**(WY-B-20) | I | $CO_2CH_2CF_3$ | 12 | 39 | 47 | 2000 | 122 | 3000 |
| **28c**(iodo-βCCE) | I | $CO_2Et$ | 4.8 | 31 | 34 | 1000 | 286 | 1000 |
| **28d**(WY-B-08) | I | $CO_2CH(CF_3)_2$ | 78 | 301 | 131 | 3000 | 681 | 3000 |
| **29a**(WYS13) | (furanyl-O) | $CO_2tBu$ | 2.4 | 13 | 27.5 | NA | 163 | 5000 |
| **29b**(WYB27-I) | (furanyl-O) | $CO_2CH_2CF_3$ | 26 | 143 | 117 | 3000 | 127 | 2000 |
| **30a**(WYS12) | (thienyl-S) | $CO_2tBu$ | 37 | 166 | 314 | NA | 2861 | 5000 |
| **30b**(WYB27-2) | (thienyl-S) | $CO_2CH_2CF_3$ | 9.2 | 13 | 72 | 2000 | 449 | 2000 |
| **31a**(WYS15) | (phenyl) | $CO_2tBu$ | 3.63 | 2.02 | 44.3 | NA | 76.5 | 5000 |
| **31b**(WYB29-2) | (phenyl) | $CO_2CH_2CF_3$ | 25 | 137 | 125 | 2000 | 299 | 2000 |
| CMA57 | F | $COC_3H_7$ | 3.7 | 27 | 40 | NA | 254 | >2500 |
| CM-A-82a | $C(CH_3)_3$ | $CO_2tBu$ | 2.78 | 8.93 | 24.5 | 1000 | 7.49 | 1000 |
| CM-A-87 | F | $CO_2tBu$ | 1.62 | 4.54 | 14.7 | 1000 | 4.61 | 1000 |
| **32**(WYS2) | Bcct≡Bcct | | 30 | 124 | 100 | >300 | >300 | >4000 |
| **33**(WYS6) | Bcct≡≡Bcct | | 120 | 1059 | 3942 | 5000 | 5000 | 5000 |

The affinity of compounds at GABA_A/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam binding to HEK cell membranes expressing human receptors of composition $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_4\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, and $\alpha_6\beta_3\gamma_2$.[119] Data represent the average of at least three determinations with a SEM of ±5%. The most selective ligands are in bold type.

Harvey et al.[2,64] In comparison to other BzR antagonists such as flumazenil and ZK 93426, as mentioned, βCCt exhibited either a neutral or low-efficacy agonist response at GABA $\alpha_1$ (96 ± 7%), $\alpha_2$ (99 ± 10%), $\alpha_3$ (108 ± 6%), and $\alpha_4$ (107 ± 5%) receptors. However, a low-efficacy partial inverse agonist response was observed at the $\alpha_5$ receptor (88 ± 7% of the GABA response). Flumazenil exhibited

an efficacy profile that was qualitatively similar to βCCt at the $\alpha_1$ (99 ± 5%), $\alpha_3$ (118 ± 7%), and $\alpha_5$ (96 ± 6%) subtypes. At the $\alpha_2$ receptor, flumazenil produced a low-efficacy agonist response (115 ± 4%), while βCCt was GABA neutral (99 ± 10%). Flumazenil also produced a qualitatively similar response to βCCt at the $\alpha_4$ receptor, albeit the magnitude of GABA potentiation by flumazenil
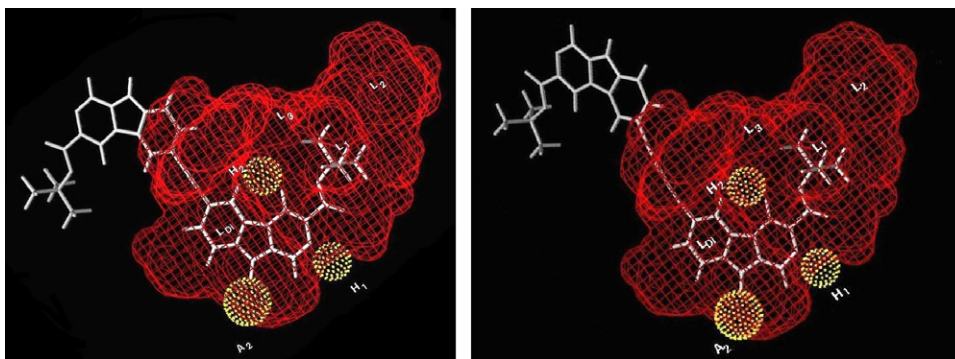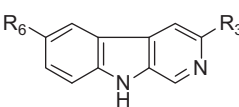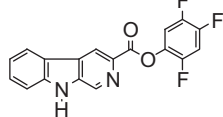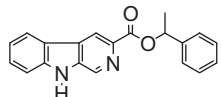
**Figure 4.** Alignment of bivalent ligands **32** and **33** within the included volume of the $\alpha1\beta3\gamma2$ subtype.

**Table 2a**
Affinities ($K_i$ = nM) of 3-substituted $\beta$-carbolines at $\alpha_x\beta_3\gamma_2$ ($x$ = 1–3, 5, 6) receptor subtypes



| Ligands | $R_6$ | $R_3$ | $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_4$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|---|---|---|---|
| **BCCE** | **H** | **$CO_2Et$** | **1.2** | **4.9** | **5.7** | **1000** | **26.8** | **2700** |
| **15** | H | $CO_2CH_2CF_3$ | 3.0 | 24.5 | 41.7 | >500 | 125.7 | >2000 |
| **16**(WYB09-1) | H | $CO_2CH(CF_3)_2$ | 3.99 | 8 | 32 | 1000 | 461 | 2000 |
| **17**(WYB23-1) | H | $CO_2CH_2CCl_3$ | 10 | 33 | 43 | 1000 | 189 | 2000 |
| **18**(WYB17) | H | $CO_2CH(CH_3)CCl_3$ | 2000 | 2000 | 2000 | 3000 | 2000 | 5000 |
| **19**(CMA64) | H | $CO_2CH(CH_3)C_2H_5$ | 18 | 60 | 116 | NA | 216 | >2000 |
| **20**(CMA69) | H | $CO_2CH(CF_3)C_2H_5$ | 1000 | 1000 | 1000 | NA | 1000 | >2000 |
| **25**(WY-B-24) | | | 22.0 | 177 | 44.8 | 3000 | 422 | 3000 |
| **26**(CM-A-77) | | | 33.5 | 1000 | 1000 | 1000 | 1000 | 3000 |

The affinity of compounds at $GABA_A$/BzR recombinant subtypes was measured by competition for [$^3$H]flunitrazepam binding to HEK cell membranes expressing human receptors of composition $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_4\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, and $\alpha_6\beta_3\gamma_2$.[119] Data represent the average of at least three determinations with a SEM of ±5%. The most selective ligands are in bold type.

far exceeded that of $\beta$CCt (132 ± 6% vs 108 ± 6%, respectively). However, it is important to note, with regard to $\alpha_4/\alpha_6\beta_3\gamma_2$ subtypes, the agonist effect was observed at 10 μM, far above that required for agonist efficacy at the DS subtypes. In contrast, ZK 93426 produced a clear agonist profile, potentiating GABAergic activity by 137 ± 8−148 ± 11% across the $\alpha_1$–$\alpha_4$ subtypes, but was GABA neutral at the $\alpha_5$ receptor (96 ± 6%). These findings suggested that $\beta$CCt had no appreciable intrinsic efficacy. The rationale for referring to this agent as a 'mixed agonist–antagonist' was based on the fact that, despite the ability to potentiate GABA at certain receptor subtypes, it was 'GABA neutral' at select doses. In addition, at select doses, $\beta$CCt and 3-P$\beta$C were capable of competitive antagonism of classical benzodiazepine agonists,[60,64,72] therefore, the development of subtype-selective antagonists for $GABA_A$ receptors, such as $\beta$CCt, which targeted the $GABA_A$ $\alpha_1$ receptor as a weak agonist–like antagonist,[74] should facilitate efforts to understand the anti-alcohol action of $\beta$-carbolines in nonhuman primates and humans as well.
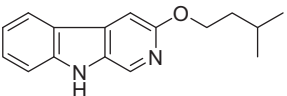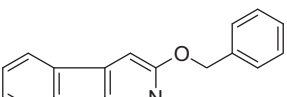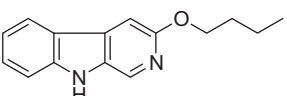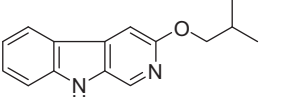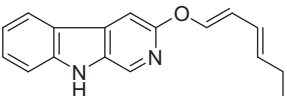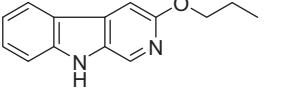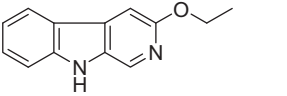
In the NIMH supported PDSP screen neither $\beta$CCT, 3PBC, nor WYS8 exhibited significant interactions at other receptors (see http://pdsp.med.unc.edu for details).

## 5. Conclusion

Ethanol allosterically modulates the GABA receptor complex to open the chloride channel and hyperpolarize cells. At the pharmacological level, the effects of ethanol can be antagonized with GABA antagonists.[109] Unfortunately, the paucity of high affinity subtype selective ligands capable of discriminating among the various $GABA_A$ receptor subtypes has, thus far, precluded study of the precise role $GABA_A$ subunits play in mediating EtOH-maintained responding.

A series of $\beta$-carboline ligands described here has exhibited some selectivity at the $\alpha_1$ receptor subtype which included $\beta$-carboline-3-carboxylate-$t$-butyl ester ($\beta$CCt, **1**) and 3-propoxy-$\beta$-carboline hydrochloride (3-PBC, **2**). These ligands displayed a 20-fold and 10-fold selectivity, respectively, for the $\alpha_1$ subtype over the $\alpha_2$ and $\alpha_3$ receptors, as well as over 150-fold selectivity for the $\alpha_1$ site over the $\alpha_5$ subtype.[2,3] $\beta$CCt (**1**) was more selective at the $\alpha_1$ subtype in vitro than the classical $\alpha_1$ selective agonists zolpidem (**3**) and CL 218872 (**4**).[60,98,99] $\beta$CCt and 3-PBC are capable of competitive antagonism of classical benzodiazepine agonists,[3,60,72] therefore, the development of subtype-selective antagonists for

**Table 2b**
Affinities ($K_i$ = nM) of ether-substituted β-carbolines at $\alpha_x\beta_3\gamma_2$ ($x$ = 1–3, 5, 6) receptor subtypes

| Ligands | $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_5$ | $\alpha_6$ |
|---|---|---|---|---|---|
| **34** | 350.2 | 3000 | 3000 | 3000 | 10,000 |
| **35** | 830 | 3000 | 3000 | 10,000 | 10,000 |
| **36** | 36.9 | 194 | 245 | 1000 | 1000 |
| **37** | 24.9 | 123.6 | 139.2 | 1000 | 10,000 |
| **38** | 245 | 818 | 859 | 10,000 | 10,000 |
| **2** | 5.3 | 52.3 | 68.8 | 591 | 1000 |
| **39** | 6.43 | 25.1 | 28.2 | 826 | 1000 |

The affinity of compounds at GABA$_A$/BzR recombinant subtypes was measured by competition for [$^3$H]flunitrazepam binding to HEK cell membranes expressing human receptors of composition $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_4\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, and $\alpha_6\beta_3\gamma_2$.[119] Data represent the average of at least three determinations with a SEM of ±5%.

GABA$_A$ receptors which targets the GABA$_A$ $\alpha_1$ receptor as a weak agonist–like antagonist,[74] should facilitate efforts to understand the anti-alcohol actions of β-carbolines in nonhuman and human primates alike. Compared with Naltrexone, the reductions in alcohol responding were more selective and longer in duration.[107] The goal of the present study was to identify novel $\alpha_1$ GABA$_A$ subtype-preferring ligands that may serve as prototypes for further evaluation of clinical efficacy. These types of compounds may provide treatments for excessive alcohol drinking and the negative affective states associated with abstinence. Ligands that enhance GABAergic tone may be effective and safe treatments for both excessive alcohol drinking and the negative affective states associated with abstinence. This may represent novel, new pharmacotherapies to treat alcoholism.

Studies of the structure–activity relationships confirmed that occupation of region L$_{Di}$ of the receptor pharmacophore model

did enhance $\alpha_1$ selectivity in comparison to the affinity of the non-selective ligands or the $\alpha_5$ selective ligands. Full occupation of the L$_{Di}$ lipophilic region by β-carbolines may account for the potency/selectivity of this class of ligands at the $\alpha_1$ subunit. Based on the SAR, the most potent $\alpha_1$ selective ligand was 6-substituted acetylenyl βCCt (WYS8, **7**). It was suggested the attenuation of EtOH-motivated responding effected by WYS8 (**7**) was mediated via the $\alpha_1$ selective antagonism of the GABA$_A$/BzR receptor.[2] In regard to ester functions at C(3), although both (R) and (S) enantiomers exhibited approximately a fourfold selectivity for the $\alpha_1\beta_3\gamma_2$ subtype, the (R) isomer remained more potent in vitro at all five BzR sites. Two factors affected high affinity binding at BzR with respect to β-carbolines, one of which was the lipophilicity of the substituent which interacted at L$_1$. The second factor was the ability of the substituent at position-3 to release electron density to the pyridine ring.

The most selective ligand for $\alpha_1$ subtypes, to date, to these authors' knowledge was WYS8 (**7**). This 6-substituted acetylenyl βCCt (**7**) was 214-fold more selective for α1 isoforms over $\alpha_5$ isoforms. WYS8 can clearly be differentiated from non-selective BDZs by its selective binding affinity at the $\alpha_1$ receptor subunit and reduced capacity to potentiate GABA in Xenopus oocytes.

Innate elevations of the $\alpha_1$ and $\alpha_2$ subunits of the HAD rat may contribute to the capacity of novel β-carboline ligands to function as both anxiolytic agents and alcohol antagonists in this genetic rat line.[107] These differences may explain the capacity of these novel β-carboline ligands to block alcohol drinking and still exhibit anxiolytic actions in the P and HAD alcoholic rats. WYS8 may be a suitable ligand to evaluate as a preclinical agent to reduce alcohol dependence. Its reduced efficacy at the $\alpha_{1-2}$ subunits in potentiating GABA may render it a safe BDZ receptor ligand devoid of synergistic interactions with alcohol.

## 6. Experimental section
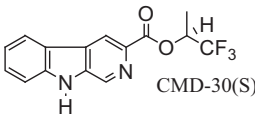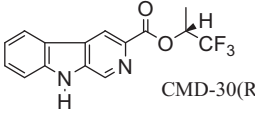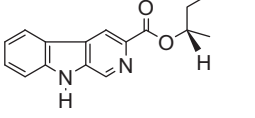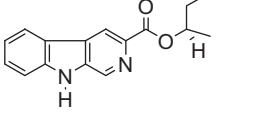
### 6.1. Biological and pharmacological testing

Methods for in vitro receptor binding and efficacy in oocytes follow previous work.[129,130] Competition binding assays were performed in a total volume of 0.5 mL at 4 °C for 1 h using [$^3$H] flunitrazepam as the radiolabel. For these binding assays, 20–50 µg of membrane protein harvested with hypotonic buffer (50 mM Tris–acetate pH 7.4 at 4 °C) was incubated with the radiolabel as previously described.[119] Nonspecific binding was defined as radioactivity bound in the presence of 100 µM diazepam and represented less than 20% of total binding. Membranes were harvested with a Brandel cell harvester followed by three ice-cold washes onto polyethyleneimine-pretreated (0.3%) Whatman GF/C filters. Filters were dried overnight and then soaked in Ecoscint A liquid scintillation cocktail (National Diagnostics; Atlanta, GA). Bound radioactivity was quantified by liquid scintillation counting. Membrane protein concentrations were determined using an assay kit from Bio-Rad (Hercules, CA) with bovine serum albumin as the standard.

The results are summarized in Tables 1–3.

The electrophysiological analyses of all selective compounds were performed with whole cell variation of the patch-clamp-technique, in HEK cells employing GABA concentrations around the subtype-specific EC$_{20}$[3,120] to depict the quantitative efficacy difference (i.e., GABA modulation) and qualitative subunit modulation (i.e., subunit type) of these ligands relative to diazepam.
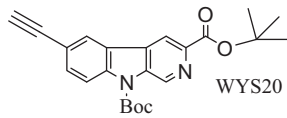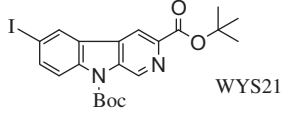
Melting points were taken on a Thomas–Hoover melting point apparatus or an Electrothermal Model IA8100 digital melting point apparatus and are reported uncorrected. Proton NMR spectra were recorded on a Bruker 250- or 300-MHz multiple-probe

**Table 3a**
Affinities ($K_i$ = nM) of chiral 3-substituted β-carbolines at $\alpha_x\beta_3\gamma_2$ ($x$ = 1–3, 5, 6) receptor subtypes

| Ligands | | $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_4$ | $\alpha_5$ | $\alpha_6$ |
|---------|---|------------|------------|------------|------------|------------|------------|
| 21 | CMD-30(S) | 90 | 931 | 172 | >3000 | 1847 | >2000 |
| 22 | CMD-30(R) | 27.0 | 343.3 | 453 | >3000 | 1847 | >2000 |
| 23 | | 17 | 59 | 88 | NA | 1444 | >3000 |
| 24 | | 7.7 | 32.5 | 43 | NA | 69 | >2000 |

The affinity of compounds at GABA$_A$/BzR recombinant subtypes was measured by competition for [³H]flunitrazepam binding to HEK cell membranes expressing human receptors of composition $\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_4\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, and $\alpha_6\beta_3\gamma_2$.[119] Data represent the average of at least three determinations with a SEM of ±5%.

**Table 3b**
Affinities ($K_i$ = nM) of Boc protected 3-substituted β-carbolines at $\alpha_x\beta_3\gamma_2$ ($x$ = 1–3, 5, 6) receptor subtypes

| Ligands | | $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_4$ | $\alpha_5$ | $\alpha_6$ |
|---------|---|------------|------------|------------|------------|------------|------------|
| 40 | WYS20 | 450 | 5000 | ND | ND | 5000 | 5000 |
| 41 | WYS21 | ND | ND | ND | ND | 1847 | ND |

ND = not determined yet (see previous tables for details of receptor binding). Data represent the average of at least three determinations with a SEM of ±5%.

instrument. Infrared spectra were recorded on a Nicolet DX FTIR BX V5.07 spectrometer or a Mattson Polaris IR-10400 instrument. Low-resolution mass spectral data (EI/CI) were obtained on a Hewlett-Packard 5985B GC-mass spectrometer, while high resolution mass spectral data were taken on a VG autospectrometer (Double Focusing High Resolution GC/Mass Spectrometer, UK). Microanalyses were performed on a CE Elantech EA1110 elemental analyzer. Analytical TLC plates employed were E. Merck Brinkman UV active silica gel (Kieselgel 60 F254) on plastic, and silica gel 60b for flash chromatography was purchased from E. M. Laboratories. All chemicals were purchased from Aldrich Chemical Co. unless otherwise stated. All solvents were dried according to the published procedures.

### 6.1.1. 1,2,3,4-Tetrahydro-9H-pyrido[3,4-b]indole-3-carboxylic acid (11)

D,L-Tryptophan (1000 g, 4.9 mol) was added to a solution of aq sodium hydroxide (12 L, 0.4 N) after which the mixture was stirred until it dissolved. Formaldehyde (560 mL of a 37% aq solution, 6.9 mol) was added and the solution was allowed to stir for three days at 37 °C. Glacial acetic acid (400 mL) was added which resulted in the precipitation of a solid as a fine suspension. The mixture was allowed to stir for two days, after which additional solid formed. The solid was filtered from the medium, washed with water (4 × 1000 mL), and dried to give **11** (953 g, 90.0%). Compound **11**: mp 295 °C (lit. mp 293 °C)[135] (lit. mp 286 °C);[136] (IR (KBr) 3600–2300, 1630 cm⁻¹; MS (CI, CH$_4$), $m/z$ (relative intensity) 217 (M⁺+1, 50), 216 (62), 169 (59), 144 (100). This material was employed directly in the next step.

### 6.1.2. Ethyl 1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole-3-carboxylate (12)

The 1,2,3,4-tetra-hydro-9H-pyrido[3,4-b]indole-3-carboxylic acid **11** (500 g, 2.3 mol) was dissolved in anhydrous ethanol (9 L) in a 12 L (three neck) flask, and concd sulfuric acid (98%, 245 mL, 4.6 mol) was carefully added to the solution until most of the solid dissolved. The reaction mixture was heated to reflux under nitrogen until the starting material was no longer detected by TLC on silica gel (48 h), and the solution became homogeneous. The reaction solution was cooled and the solvent removed under reduced pressure. The residue was dissolved in H$_2$O (6.4 L) and the pH of the solution adjusted to 8 with cold aq NH$_4$OH (concd) after which a precipitate formed. This mixture was then extracted with CHCl$_3$ (6 × 2.5 L). The combined organic layers were dried (Na$_2$SO$_4$) and the solvent was removed under reduced pressure to yield a light tan solid which was dried in a vacuum oven at 100 °C to provide **12** (465 g, 83%). Compound **11**: mp 150 °C (lit. mp 149–150 °C);[90,137] ¹H (300 MHz, CDCl$_3$) $\delta$ 1.33 (t, $J$ = 7.32 Hz, 3H), 2.45 (br s, 1H), 2.88 (dd, $J$ = 9.70 Hz, $J$ = 15.37 Hz, 1H), 3.13 (dd, $J$ = 4.76 Hz, $J$ = 15.5 Hz, 1H), 3.77 (dd, $J$ = 4.76 Hz, $J$ = 9.70 Hz, 1H),
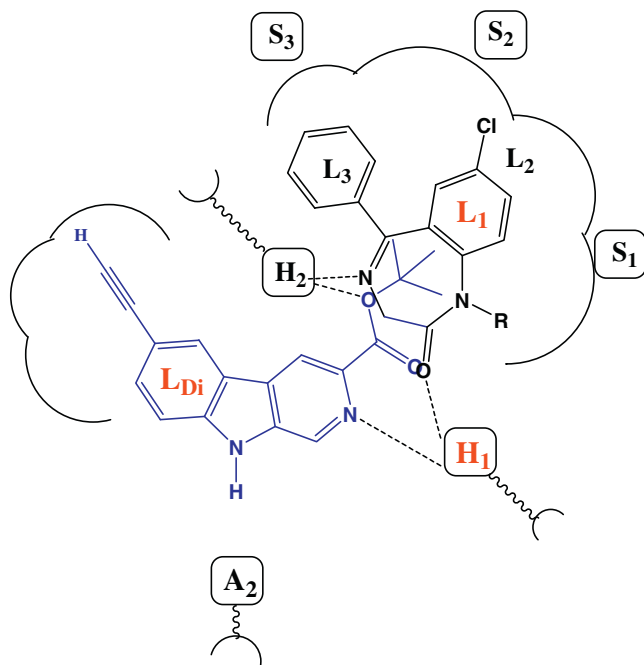
**Figure 5a.** Overlap of diazepam and βCCt in the pharmacophore/receptor model. The structure of WYS8 and diazepam in a simple representation of the pharmacophore model. WYS8 (**7**) (blue line) and diazepam (black line) fitted to the inclusive pharmacophore model for the BzR. Sites H$_1$ and H$_2$ represent hydrogen bond donor sites on the receptor protein complex, while A$_2$ represents a hydrogen bond acceptor site necessary for potent inverse activity in vivo. L$_1$, L$_2$, L$_3$, and L$_{Di}$ are four lipophilic regions in the binding pharmacophore. Descriptors S$_1$, S$_2$, and S$_3$ are regions of negative steric repulsion.

4.07 (br s, 2H), 4.26 (q, *J* = 7.14 Hz, 2H), 7.18–7.08 (m, 2H), 7.29 (d, *J* = 7.87 Hz, 1H), 7.48 (d, *J* = 7.68 Hz, 1H), 8.17 (br s, 1H); MS (CI CH$_4$) *m/e* 144 (97.3), 245 (M+1, 87), 244 (100), 183 (6), 171 (33), 144 (83). This material was employed directly in the next step.

### 6.1.3. Ethyl 9*H*-pyrido[3,4-*b*]indole-3-carboxylate (8)

Into a round-bottom flask (12 L) equipped with a reflux condenser and an overhead stir was added 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ethyl ester **12** (200 g, 0.86 mol) and dry benzene (8 L). The solution was allowed to heat to reflux at which time activated MnO$_2$ (200 g) was added to the flask. Additional quantities of activated MnO$_2$ were added until analysis by TLC (sil-

ica gel/ethyl acetate) indicated the absence of starting material. The hot solution was filtered through a bed of Celite to remove the MnO$_2$ and the filter cake was washed with hot benzene. The benzene layers were allowed to cool. A precipitate formed and was collected by vacuum filtration, which provided (100–120 g, 50–60%) of pure β-carboline-3-carboxylic acid ethyl ester **8** (βCCE). The benzene which remained in the filtrate was removed under reduced pressure to provide 25–35 g of additional βCCE, but as crude material. The crude material could be purified by recrystalization from ethanol. Compound **8**: mp 225–227 °C (lit. 224–229 °C);[67,137,138] $^1$H (300 MHz, DMSO-$d_6$) $\delta$ 1.36 (t, *J* = 6.95 Hz, 3H), 4.37 (q, *J* = 6.95 Hz, 2H), 7.37–7.24 (m, 1H), 7.68–7.57 (m, 2H), 8.38 (d, *J* = 7.87 Hz, 1H), 8.90 (s, 1H), 8.97 (s, 1H), 10.7 (br, 1H); MS (CI, CH$_4$) *m/e* 241 (M$^+$+1, 47), 195 (22), 168 (100), 140 (9). This material was employed directly in the next step.

### 6.1.4. β-Carboline-3-carboxylic acid (9)

β-Carboline-3-carboxylic acid ethyl ester **8** (30.0 g, 0.126 mol) was suspended in aq NaOH (10%, 1.5 L) and heated to reflux until all the material had gone into solution (1 h). The heating was continued for an additional 3 h. The reaction mixture was cooled to rt and acidified by addition of ice-cold aq concd HCl to pH 4. The precipitate which resulted was stirred overnight. The solid was collected by vacuum filtration and washed with H$_2$O (2 × 150 mL). The product was dried at 80 °C under vacuum for 24 h to provide **9** (26.1 g, 99%). Compound **9**: mp 220–221 °C (lit. mp 220 °C);[67] IR (KBr) 3260, 2970, 1710 cm$^{-1}$; $^1$H NMR (CDCl$_3$) $\delta$ 7.31 (t, *J* = 7.32 Hz, 1H), 7.69–7.57 (m, 2H), 8.38 (d, *J* = 7.87 Hz, 1H), 8.90 (s, 1H), 8.96 (s, 1H), 12.10 (s, 1H); MS (CI, CH$_4$), *m/e* (M$^+$+1, 269). Anal. Calcd for C$_{16}$H$_{16}$N$_2$O$_2$ (0.55 H$_2$O): C, 69.07; H, 6.19; N, 10.07. Found: C, 68.81; H, 5.77; N, 10.00.

### 6.1.5. β-Carboline-3-carboxylic acid *t*-butyl ester (1)

To a solution of carbonyl diimidazole (28.2 g, 0.177 mol) in anhydrous DMF (1.2 L) was added dry β-carboline-3-carboxylic acid **9** (25 g, 0.118 mol) under argon. The reaction mixture was initially a pale yellow-colored suspension, but after stirring for 30 min, a purple or red-colored solution resulted. The reaction mixture was stirred for an additional 2 h at rt and carbon dioxide was released during the reaction. Analysis by TLC (silica gel) indicated the absence of starting material on the baseline. To this reaction mixture was added dry DBU (18 g, 0.118 mol) and dry freshly distilled *t*-butyl alcohol (437 g/560 mL, 50 equiv). The mixture was heated at 85 °C for 18 h until analysis by TLC indicated the disappearance of the imidazole intermediate. The solvent was then
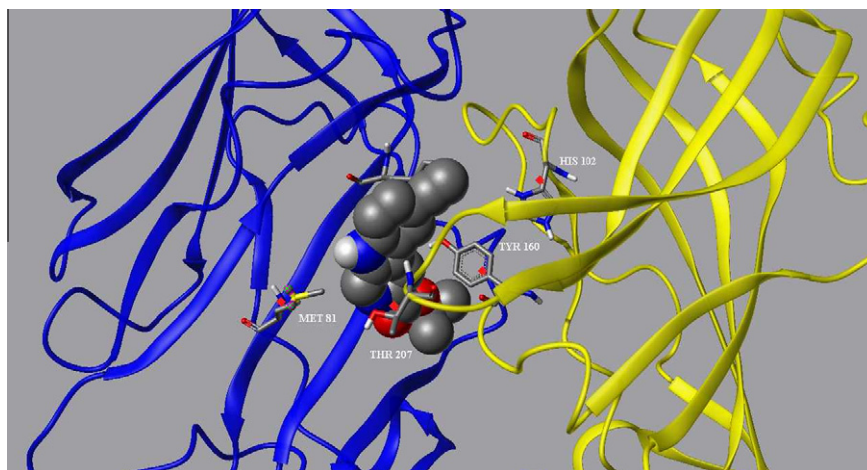


**Figure 5b.** WYS8 docked in the BzR site of the α1 subtype GABA$_A$ receptor. The α1 and γ2 subunits are rendered in yellow and blue, respectively.

removed under reduced pressure. The residue was partitioned between $CH_2Cl_2$ (1.2 L) and $H_2O$ (800 mL). The organic layer was separated and the $H_2O$ layer was extracted with $CH_2Cl_2$ (2 × 500 mL). The combined organic layer was washed with an aq solution of 10% $K_2CO_3$, water, brine and dried ($Na_2SO_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 1:1) to provide βCCt (20 g, 65%) as a white solid. βCCt can be recrystallized from EtOAc to provide white crystals **1**: mp 301–303 °C (lit. mp 298–300);[80] IR (KBr) 3500–3400, 3200–3000, 1610, 1560, 1370, 1340 cm$^{-1}$; $^{1}$H (300 MHz, DMSO-$d_6$) δ 1.74 (s, 9H), 7.37 (t, J = 7.48 Hz, 1H), 7.63 (t, J = 7.66 Hz, 1H), 7.73 (d, J = 8.52 Hz, 1H), 8.24 (d, J = 7.89 Hz, 1H), 8.85 (s, 1H), 9.12 (s, 1H), 8.97 (s, 1H), 10.19 (br, 1H); The spectral data for **1** were identical to those reported in the literature.[88]

### 6.1.6. 2,2,2-Trifluoroethyl β-carboline-3-carboxylate 15

Compound **15** was prepared from β-carboline-3-carboxylic acid **9** and 2,2,2-trifluoroethyl alcohol following the procedure employed for the preparation of **1**. Compound **15**: mp 264–266 °C; IR (NaCl) 3275, 1735 cm$^{-1}$; $^{1}$H NMR (300 MHz, CDCl$_3$) 4.87 (m, 2H), 7.42 (m, 1H), 7.65 (m, 2H), 8.24 (d, J = 7.9 Hz, 1H), 8.93 (s, 1H), 9.09 (s, 1H), 9.10 (br s, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) δ 60.4, 60.8, 112.8, 118.9, 120.7, 121.2, 122.7, 127.8, 129.2, 134.5, 135.2, 138.1, 141.3, 164.5; MS (EI) *m/e* (relative intensity) 294 (M$^+$, 30), 195 (7), 168 (100), 167 (42). Anal. Calcd for $C_{14}H_9F_3N_2O_2$: C, 57.15; H, 3.08; N, 9.52. Found: C, 57.22; H, 3.14; N, 9.23.

### 6.1.7. 9H-β-Carboline-3-carboxylic acid 2,2,2-trifluoro-1-trifluoromethyl-ethyl ester 16

Compound **16** was prepared following the procedure employed for the preparation of **1**. Compound **16**: $^{1}$H NMR (300 MHz, DMSO-$d_6$) δ 7.05–7.21 (m, 1H), 7.33–7.44 (t, 1H), 7.61–7.72 (m, 2H), 8.48 (d, J = 7.89 Hz, 1H), 9.09 (s, 2H), 12.31 (s, 1H); $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) δ 65.9, 66.8, 112.9, 120.1, 120.9, 122.8, 127.8, 129.4, 133.3, 134.9, 138.4, 141.3, 162.9. This material was pure by TLC (silica gel).

### 6.1.8. 9H-β-Carboline-3-carboxylic acid 2,2,2-trichloro-ethyl ester 17

Compound **17** was prepared following the procedure employed for the preparation of **1**. Compound **17**: $^{1}$H NMR (300 MHz, CDCl$_3$) δ 5.17 (s, 2H), 7.38–7.44 (m, 1H), 7.63–7.74 (m, 2H), 8.27 (d, J = 7.95 Hz, 1H), 8.95 (s, 1H), 9.21 (s, 1H), 9.66 (s, 1H). This material was pure by TLC (silica gel).

### 6.1.9. 9H-β-Carboline-3-carboxylic acid 2,2,2-trifluoro-1-methyl-ethyl ester 18

Compound **18** was prepared following the procedure employed for the preparation of **1**. Compound **18**: mp 247–249 °C; IR (NaCl) 2359, 1729, 1345, 1251, 1092, 729, 450 cm$^{-1}$; $^{1}$H NMR (300 MHz, CDCl$_3$) δ 1.82 (d, J = 6.1 Hz, 3H), 5.96 (q, 1H), 7.42 (t, 1H), 7.67 (m, 2H), 8.26 (d, J = 7.6 Hz, 1H), 8.8 (s, 1H), 8.9 (s, 1H), 9.5 (br, s, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) δ 16.5, 79.1, 112.3, 118.5, 121.2, 121.5, 121.9, 128.8, 129.2, 133.9, 137.4. This material was pure by TLC (silica gel).

### 6.1.10. (S)-1,1,1-Trifluoroisopropyl β-carboline-3-carboxylate (21)

To a solution of carbonyl diimidazole (0.168 g, 1.03 mmol) in anhydrous DMF (5 mL), β-carboline-3-carboxylic acid **9** (0.10 g, 0.47 mmol) was added. The reaction mixture which resulted was stirred for 2 h at rt until analysis by TLC (silica gel) indicated the absence of starting material on the baseline. The solution which resulted was then cooled to −6 °C and this was followed by addition of (S)-1,1,1-trifluoropropan-2-ol (2.3 equiv) which was contaminated with some EtOH. The dry DBU (100 mg, 0.68 mmol) in dry

DMF (0.5 mL) was slowly syringed into the reaction mixture at −6 °C. The mixture was stirred at 0 °C for 8 h until analysis by TLC (silica gel) indicated the disappearance of the imidazole intermediate. The reaction mixture was then poured into ice water (30 mL) and extracted with $CH_2Cl_2$ (3 × 40 mL). The combined organic layers were washed with $H_2O$ (5 × 40 mL), brine and dried ($Na_2SO_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexanes = 2:1) to provide **21** (0.113 g, 78%) as a white solid. Compound **21**: mp 239–241 °C; $[\alpha]_D^{27} = -9.62$ (c 0.81, in CHCl$_3$); IR (NaCl) 3266, 1725, 1502 cm$^{-1}$; $^{1}$H NMR (300 MHz, CDCl$_3$) δ 1.63 (d, J = 6.6 Hz, 3H), 5.75 (m, 1H), 7.40 (t, J = 7.5 Hz, 1H), 7.64 (t, J = 7.6 Hz, 1H), 7.72 (d, J = 8.1 Hz, 1H), 8.25 (d, J = 7.9 Hz, 1H), 8.91 (s, 1H), 9.18 (s, 1H), 10.04 (br s, 1H); MS (EI) *m/e* (relative intensity) 308 (M$^+$, 17), 168 (100), 140 (21). Exact mass Calcd for $C_{15}H_{11}F_3N_2O_2$: 308.0773. Found: 308.0773. Anal. Calcd for $C_{15}H_{11}F_3N_2O_2$: C, 58.45; H, 3.60; N, 9.09. Found: C, 58.15; H, 3.63; N, 8.88.

### 6.1.11. (S)-1,1,1-Trifluorobutan-2-ol

(S)-1,1,1-Trifluorobutan-2-ol was prepared following the literature procedure.[114] To an oven-dried, 25 mL round-bottom flask was transferred (−)-DIP-Chloride (10.68 g, 33 mmol) in a glove box. Then 1,1,1-trifluorobutan-2-one (4.03 g, 32 mmol) was added at rt under argon. The reaction mixture was stirred at rt for 10 h. Ethyl ether was added and the reaction solution was cooled to 0 °C followed by addition of acetaldehyde (1.6 g, 1.1 equiv). The reaction mixture was allowed to warm to rt and stirring was continued for 24 h while the second equivalent of α-pinene was liberated. An aq solution of sodium hydroxide (2.5 M, 30 mL) was added and the solution which resulted was extracted with ether (3 × 30 mL). The ether layer was dried ($Na_2SO_4$) and fractionally distilled through a Pyrex distilling column packed with glass beads. The desired alcohol was collected along with EtOH (20%). (S)-1,1,1-Trifluorobutan-2-ol: $^{1}$H NMR (300 MHz, CDCl$_3$) 1.04 (dt, J = 0.6 Hz and J = 7.4 Hz, 3H), 1.58 (m, 1H), 1.72 (m, 1H), 3.82 (m, 1H). This material was used in a later step without further purification.

### 6.1.12. (R)-1,1,1-Trifluoroisopropyl β-carboline-3-carboxylate 22

Compound **22** was prepared from the acid **9** and (R)-1,1,1-trifluoropropan-2-ol following the procedure employed for preparation of (S)-1,1,1-trifluoro-propan-2-ol **21**. Compound **22**: mp 239–241 °C; $[\alpha]_D^{27} = 8.73$ (c 0.88, in CHCl$_3$); The spectral data for **22** were identical to those for **21**; however, the optical rotation was in the opposite direction.

### 6.1.13. (S)-β-Carboline-3-carboxylic acid sec-butyl ester (23)

To a solution of carbonyl diimidazole (1.53 g, 9.4 mmol) in anhydrous DMF (50 mL) was added β-carboline-3-carboxylic acid **9** (1.0 g, 4.7 mmol). The reaction mixture was stirred for 2 h at rt and carbon dioxide was released during the reaction. Analysis by TLC (silica gel) indicated the absence of starting material on the baseline. To this reaction mixture was added dry DBU (0.72 g, 4.7 mmol) and dry (S)-butyl alcohol (1.13 g, 15.2 mmol). The mixture which resulted was heated at 55 °C for 8 h until analysis by TLC (silica gel) indicated the disappearance of the imidazole intermediate. The solvent was then removed under reduced pressure. The residue was partitioned between $CH_2Cl_2$ (100 mL) and $H_2O$ (100 mL). The organic layer was separated and the $H_2O$ layer was extracted with $CH_2Cl_2$ (2 × 80 mL). The combined organic layer was washed with $H_2O$ (3 × 100 mL), brine and dried ($Na_2SO_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 2:1) to provide **23** (0.96 g, 76%) as a white solid. Compound

**23**: mp 212–213 °C; $[\alpha]_{25}$ = 35.6 (CHCl$_3$, *c* 1.43); IR (KBr) 3222, 1706, 1622, 1494 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.99 (t, *J* = 7.5 Hz, 3H), 1.40 (d, *J* = 6.3 Hz, 3H), 1.79 (m, 2H), 5.27 (m, 1H), 7.34 (t, *J* = 7.9 Hz, 1H), 7.57 (t, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 8.3 Hz, 1H), 8.21 (d, *J* = 7.9 Hz, 1H), 8.44 (s, 1H), 9.10 (s, 1H), 11.47 (br s, 1H); MS (CI, CH$_4$) *m/e* (relative intensity) 269 (M$^+$+1, 100), 241 (15), 213 (41), 195 (14). Anal. Calcd for C$_{16}$H$_{16}$N$_2$O$_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.33; H, 6.09; N, 10.26.

### 6.1.14. (*R*)-β-Carboline-3-carboxylic acid sec-butyl ester 24

Compound **24** was prepared in 75% yield following the procedure for preparation of **1**. Compound **24**: $[\alpha]_{25}$ = −35.2 (CHCl$_3$, *c* 1.25). The spectral data for **22** were identical to those for **23**, except the optical rotation was opposite in direction.

### 6.1.15. 6-Iodo-9*H*-β-carboline-3-carboxylic acid *t*-butyl ester (5)

Into a round-bottom flask (250 mL) was added CF$_3$CO$_2$Ag (1.03 g, 4.67 mmol), β-carboline-3-carboxylic acid *t*-butyl ester **9** (1.02 g, 3.83 mmol), and CHCl$_3$ (100 mL). This was followed by addition of iodine (1.15 g, 4.66 mmol). The reaction mixture was allowed to stir at rt for 6 h after which another portion of CF$_3$CO$_2$Ag (500 mg, 2.26 mmol) was added and stirring continued for another 10 h at reflux. Analysis by TLC (silica gel) indicated that most of the βCCt had disappeared. The reaction mixture was filtered through a bed of Celite to remove the solid salts and the filter cake was washed with EtOH (3 × 50 mL). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 4:1) to provide **5** (908 mg, 65%) as a white solid. Compound **5**: mp 347–348 °C (dec); IR (NaCl) 3223, 1710, 1485, 1323, 1245, 1161, 1104, 1020 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.68 (s, 9H), 7.63 (d, *J* = 8.6 Hz, 1H), 7.85 (dd, *J* = 1.5 Hz and *J* = 8.6 Hz, 1H), 8.12 (s, 1H), 8.53 (d, *J* = 1.4 Hz, 1H), 9.31 (s, 1H), 11.47 (br s, 1H); $^{13}$C NMR (75.7 MHz, CDCl$_3$) $\delta$ 28.3, 80.8, 83.6, 115.1, 118.1, 123.9, 126.7, 131.2, 134.1, 136.8, 137.5, 138.7, 140.4, 164.9; MS (EI800) *m/e* (relative intensity) 395 (M$^+$+1, 18), 367 (20), 339 (100), 268 (33). Anal. Calcd for C$_{16}$H$_{15}$IN$_2$O$_2$: C, 48.75; H, 3.84; N, 7.11. Found: C, 49.01; H, 3.91; N, 6.95.

### 6.1.16. 6-Trimethylsilanylethynyl-9*H*-β-carboline-3-carboxylic acid *t*-butyl ester (6)

Into a round-bottom flask (50 mL) which contained a solution of degassed THF/Et$_3$N (10 mL/2 mL), was added 6-iodo-9*H*-β-carboline-3-carboxylic acid *t*-butyl ester **5** (400 mg, 1.02 mmol), bis[triphenylphosphine] palladium dichloride (35 mg, 5 mol %) and copper(I) iodide (7 mg, 5 mol %). *Note*: Practically, on a small scale, CuI could be used up to 10–15 mol % because of its lower molecular weight compared to the palladium catalyst; on a bigger scale, both Pd(PPh$_3$)$_2$Cl$_2$ and CuI can be used as low as 0.5–1 mol %. The reaction mixture was then degassed two times with an oil pump at −78 °C, and then the trimethylsilyl acetylene (300 mg, 3.06 mmol) was added into the mixture and it was degassed one more time at −78 °C. The reaction mixture was gradually allowed to warm to rt and stirred at rt for an additional 0.5–1 h until all the starting material had disappeared (TLC analysis indicated that the original red spot changed color to purple, since both s.m. and product had very similar $R_f$ values). The solvent was removed under reduced pressure at this point and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 4:1) to provide **6** (340 mg, 92%) as a white solid. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.71 (s, 9H), 1.75 (s, 9H), 7.67 (m, 2H), 8.26 (d, *J* = 7.6 Hz, 1H), 8.8 (s, 1H), 8.9 (s, 1H), 9.5 (br, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 0.021, 28.3, 81.9, 92.6, 105.7, 113.2, 114.9, 117.7, 121.3, 125.5, 128.3, 132.4, 133.8, 138.2, 138.4, 141.5, 165.4; EIMS 364 (M$^+$, 38), 293 (12), 264 (100), 249 (48), 124 (60). This material was employed directly in the next step.

### 6.1.17. 6-Ethynyl-9*H*-β-carboline-3-carboxylic acid *t*-butyl ester (7)-WYS8

To a solution of 6-trimethyl-silanyl-β-carboline-3-carboxylic acid *t*-butyl ester **6** (850 mg, 2.32 mmol) in THF (10 mL) was added 1.2 equiv of TBAF (2.8 mL of 1 M TBAF/THF solution) at 0 °C and then the solution was allowed to warm to rt. After consumption of the starting material as indicated by TLC, H$_2$O (10 mL) was added and the mixture was extracted with CH$_2$Cl$_2$ (3 × 20 mL). The combined organic layer was concentrated under reduced pressure and the residue was chromatographed on a short silica gel column (EtOAc/hexane = 4:1) to give **7** (620 mg, 92%) as a white solid. Compound **7**: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.75 (s, 9H), 3.54 (s, 1H), 7.62–7.85 (m, 2H), 8.41 (s, 1H), 8.83 (s, 1H), 9.33 (s, 1H), 11.1 (s, 1H); EIMS 292 (M$^+$, 25), 236 (12), 192 (100), 164 (30). This material was pure by TLC (silica gel) and used directly in a later step.

### 6.1.18. 6-Thiophen-2-yl-9*H*-β-carboline-3-carboxylic acid *t*-butyl ester (30a). Representative procedure for preparation of 6-substituted β-carbolines

A solution of 6-iodo-β-carboline-3-carbo-xylic acid *t*-butyl ester **5** (265 mg, 0.67 mmol) in dry toluene (15 mL) was degassed under vacuum and purged with dry N$_2$ through the solution three times. The mixture was then heated to 140 °C under nitrogen after which tetrakis(triphenylphosphine) palladium (0) (77 mg, 0.067 mmol, 10 mol %) and 2-(tributylstannyl) thiophene (718 mg, 2.01 mmol) were added in one portion. The mixture was heated to reflux under nitrogen. After 12 h, the mixture was allowed to cool to rt and the precipitate which resulted was removed by vacuum filtration. The filtrate was concentrated under reduced pressure and the residue was treated with a saturated aq solution of NaHCO$_3$ (30 mL) and extracted with CH$_2$Cl$_2$ (3 × 25 mL). The combined extracts were washed with brine and dried (Na$_2$SO$_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 5:1) to provide a white solid **30a** (195 mg, 83%). Compound **30a**: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 2.07 (s, 9H), 7.15 (t, 1H), 7.31 (d, *J* = 3 Hz, 1H), 7.40 (d, *J* = 3 Hz, 1H), 7.89 (q, 2H), 8.44 (s, 1H), 8.89 (s, 1H), 9.32 (s, 1H), 11.2 (s, 1H). This material was pure by TLC (silica gel).

### 6.1.19. 6-Furan-2-yl-9*H*-β-carboline-3-carboxylic acid *t*-butyl ester 29a

Compound **29a** was prepared following the procedure for preparation of **30a**. Compound **29a**: $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.63 (s, 9H), 6.63 (m, 1H), 6.97 (d, *J* = 6 Hz, 1H), 7.70 (d, *J* = 9 Hz, 1H), 7.77 (s, 1H), 7.96 (d, *J* = 9 Hz, 1H), 8.73 (s, 1H), 8.90 (s, 1H), 8.96 (s, 1H), 12.1 (s, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 28.4, 80.7, 102.3, 106.3, 112.4, 113.2, 117.4, 117.8, 127.1, 127.8, 133.6, 134.1, 138, 140.6, 142.6, 154.1, 165.0, 166.0. This material was pure by TLC (silica gel).

### 6.1.20. 6-Furan-2-yl-9*H*-β-carboline-3-carboxylic acid 2,2,2-trifluoro-ethyl ester 29b

6-Furan-2-yl-9*H*-β-carboline-3-carboxylic acid 2,2,2-trifluoro-ethyl ester **29b** was prepared following the procedure for preparation of **30a**. Compound **29b**: $^1$H NMR (250 MHz, CDCl$_3$) $\delta$ 4.82–4.92 (m, 2H), 6.54 (s, 1H), 6.75 (s 1H), 7.38 (s, 1H), 7.65 (d, *J* = 10 Hz, 1H), 7.96 (d, *J* = 10 Hz, 1H), 8.5 (s, 1H), 8.93 (s, 1H), 9.13 (s, 1H), 9.59 (s, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 60.9, 105.3, 112.5, 112.8, 113.3, 117.6, 119.2, 122.3, 123.8, 125.3, 127.9, 134.7, 135.3, 138.6, 140.6, 142.7, 154.1, 164.4. This material was pure by TLC (silica gel).

### 6.1.21. 6-Iodo-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester (35)

To a solution of 6-iodo-β-carboline-3-carboxylic acid *t*-butyl ester **5** (2 g, 5.06 mmol) in anhydrous CH$_2$Cl$_2$ (30 mL), (Boc)$_2$O (1.32 g, 6.07 mmol) and DMAP(123 mg, 1.01 mmol) were added.

The reaction mixture was allowed to stir at rt for half an hour until analysis by TLC (silica gel) indicated that the starting material had been converted into the Boc protected indole **35**. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (silica gel, EtOAc/hexane = 5:95) to provide **35** (2.3 g, 92%) as a white solid. Compound **35**: mp 317–320 °C; IR (NaCl) 2975, 2917, 1728, 1457, 1343, 1238, 1154, 1119, 1031, 811 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.71 (s, 9H), 1.77 (s, 9H), 7.91 (d, $J = 8.972$ Hz, 1H), 8.22 (d, $J = 8.972$ Hz, 1H), 8.42 (s, 1H), 8.61 (s, 1H), 9.61 (s, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 28.1, 28.2, 82.1, 85.8, 87.2, 115.9, 118.5, 125.7, 129.9, 130.6, 135.7, 138.9, 143.0, 149.6, 164.1; MS (EI) $m/e$ (relative intensity) 494 (M$^+$, 42), 438 (71), 338 (100), 294 (70), 268 (8), 212 (8), 168 (35). Anal. Calcd for C$_{21}$H$_{23}$IN$_2$O$_4$ (0.1 C$_6$H$_{14}$): C, 51.60; H, 4.85; N, 5.57. Found: C, 51.84; H, 4.88; N: 5.45.

### 6.1.22. 6-Trimethylsilanylethynyl-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester (36)

Dichlorobis(triphenylphosphine)palladium(II) (140 mg; 2 mol %), and CuI (40 mg; 2 mol %) were added to a solution of 6-iodo-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester **35** (4.9 g; 10 mmol) in anhydrous THF (30 mL) and triethylamine (10 mL). The mixture was degassed, and back-filled three times with argon. Then trimethylsilyl acetylene (1.08 g; 11 mmol) was added with stirring under argon. After the mixture was allowed to stir for 1 h, the solvents were removed under vacuum and the residue was chromatographed on a short column (silica gel, hexane/CH$_2$Cl$_2$ = 7:3) to give **36** (4.36 g, 94%) as a white solid. Compound **36**: mp 334–336 °C (dec); IR (NaCl) 2974, 2137, 1732, 1559, 1476, 1469, 1368, 1343, 1309, 1247, 1156, 1109, 872, 842, 760 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.31 (s, 9H), 1.71 (s, 9H), 1.78 (s, 9H), 7.77 (d, $J = 8.97$ Hz, 2H), 8.25 (s, 1H), 8.41 (d, $J = 8.61$ Hz, 2H), 8.65 (s, 1H), 9.62 (s, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 0.11, 28.1, 28.2, 82.1, 85.8, 94.4, 104.4, 116, 116.5, 118.7, 123.5, 124.7, 131.6, 133.7, 136.2, 138.1, 139.2, 142.9, 149.7, 164.1; MS (EI) $m/e$ (relative intensity) 465 (M$^+$, 30), 409 (100), 365 (21), 308 (80), 262 (25), 249 (40). This material was pure by TLC (silica gel) and used in the next step.

### 6.1.23. 6-Ethynyl-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester 34

To a solution of 6-trimethylsilanylethynyl-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester **36** (2.14 g, 4.6 mmol) in THF (20 mL), 1.2 equiv of TBAF (5.52 mL of 1 M TBAF/THF solution) at 0 °C was added and then the solution was allowed to warm to rt. After consumption of the starting material as indicated by TLC (silica gel), H$_2$O (10 mL) was added and the mixture was extracted with CH$_2$Cl$_2$ (20 mL, 3×). The combined organic layer was concentrated under reduced pressure and the residue was chromatographed on a short column (silica gel, hexane/CH$_2$Cl$_2$ = 4:1) to give **34** (1.66 g, 92%) as a white solid. Compound **34**: mp 226–229 °C; IR (NaCl) 3303, 2978, 2346, 2232, 1734, 1622, 1560, 1463 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.54 (s, 9H), 1.72 (s, 9H), 1.79 (s, 9H), 3.17 (s, 1H), 7.79 (d, $J = 8.76$ Hz, 2H), 8.27 (s, 1H), 8.44 (d, $J = 8.94$ Hz, 2H), 8.67 (s, 1H), 9.64 (s, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 27.3, 28.1, 28.2, 67.8, 82.1, 83.0, 85.1, 85.5, 116.0, 116.7, 123.5, 124.9, 133.7, 136.2, 138.1, 139.4, 143.0, 164.2; MS (EI) $m/e$ (relative intensity) 393 (M$^+$, 32), 338 (13), 321 (26), 293 (100), 167 (28), 139 (24). Anal. Calcd for C$_{23}$H$_{24}$N$_2$O$_4$ (0.05 CH$_2$Cl$_2$): C, 69.78; H, 6.12; N, 7.06. Found: C, 69.70; H, 6.10; N, 6.81.

### 6.1.24. 1,2-Bis(9*H*-β-carboline-3-carboxylic acid *tert*-butyl ester) ethyne (32)

Dichlorobis (tri-phenylphosphine)palladium(II) (60 mg, 2 mol %) and copper iodide (16 mg, 2 mol %) were added to a mixture of 6-ethynyl-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester **34** (1.6 g, 4.1 mmol) and 6-iodo-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester **35** (2.1 g, 4.25 mmol) in THF/TEA (30 mL; 4:1). The reaction mixture which resulted was degassed, and back-filled three times with argon. The reaction mixture was then allowed to stir at rt for about 1 h until analysis by TLC (silica gel) indicated the starting materials were absent. The solution was concentrated under reduced pressure and the residue was chromatographed on a silica gel column with CH$_2$Cl$_2$ as the eluent to give 1,2-bis(β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester) ethyne (2.97 g, 95%) as a white solid: mp 305–307 °C; IR (NaCl) 2972, 2929, 1737, 1559, 1466, 1338, 1156, 1102, 823, 624 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.72 (s, 9H), 1.79 (s, 9H), 7.86 (d, $J = 8.97$ Hz, 1H), 8.32 (s, 1H), 8.47 (d, $J = 8.79$ Hz, 1H), 8.68 (s, 1H), 9.63 (s, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 28.2, 82.1, 85.8, 89.0, 116.0, 116.7, 118.6, 123.6, 124.3, 131.5, 133.3, 136.1, 138.1, 139.1, 143.0, 149.7, 164.2; MS (FAB) 759 ((M+H)$^+$, 13). Anal. Calcd for C$_{44}$H$_{46}$N$_4$O$_8$ (H$_2$O): C, 68.03; H, 6.22; N, 7.21. Found: C, 68.10; H, 6.22; N, 7.21.

The 1,2-bis(β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester) ethyne **32** (800 mg, 1.05 mmol) was added to a distilled solution of cumene (40 mL), which had been degassed. The reaction vessel was evacuated and refilled with nitrogen three times. The temperature was then brought to reflux for about 30 min until a yellow precipitate had formed. The mixture which resulted was filtered and washed with hexane to give pure dimer **32** (545 mg, 93%). Compound **32**: mp >350 °C (dec); IR (KBr) 3227, 1716, 1327, 1162, 738, 450 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO) $\delta$ 1.62 (s, 9H), 7.70–7.80 (m, 2H), 8.7 (s, 1H), 8.94 (s, 1H), 8.99 (s, 1H), 12.25 (s, 1H); $^{13}$C NMR (300 MHz, CDCl$_3$) $\delta$ 28.3, 80.8, 113.3, 114.6, 117.9, 125.8, 127.5, 131.9, 134.2, 138.0, 140.8, 164.9; MS (FAB) 559 ((M+H)$^+$, 41). This material was pure by TLC (silica gel).

### 6.1.25. 1,4-Bis(9*H*-β-carboline-3-carboxylic acid *tert*-butyl ester) buta-1,3-diyne (33)

In a round-bottom flask (200 mL), PdCl$_2$(PPh$_3$)$_2$ (58 mg, 2 mol %), CuI (16 mg, 2 mol %), and N,N-diisopropyl-ethylamine (534 mg, 4.92 mmol) were added and the mixture stirred under argon. The flask was evacuated (degassed) and refilled with argon. The THF (40 mL) and 6-ethynyl-β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester **34** (1.6 g, 4.1 mmol) were then added (under argon) to the above mixture. To this flask methyl bromoacetate (410 mg, 2.5 mmol) was added, and the reaction mixture was stirred at rt for 6–8 h. The progress of this reaction was monitored by TLC on silica gel. After the reaction was complete, 8–10 g of silica gel was added and the solvent was removed under vacuum. The solid residue (a plug) was then placed on a column and subjected to column chromatography (CH$_2$Cl$_2$) to give 1,4-bis(β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester) buta-1,3-diyne (2.08 g, 65%): $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.74 (s, 9H), 1.81 (s, 9H), 7.82 (d, $J = 8.79$ Hz, 1H), 8.34 (s, 1H), 8.48 (d, $J = 8.79$ Hz, 1H), 8.69 (s, 1H), 9.65 (s, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 28.2, 74.0, 81.2, 82.2, 86.0, 116.1, 116.9, 117.2, 123.7, 125.5, 131.3, 134.0, 136.2, 138.1, 139.6, 143.2, 149.6, 164.1; MS (FAB) 783 ((M+H)$^+$, 100).

The 1,4-bis-(β-carboline-3,9-dicarboxylic acid di-*tert*-butyl ester) buta-1,3-diyne (411 mg, 0.5 mmol) was added to a distilled solution of cumene (20 mL), which had been degassed. The reaction mixture was evacuated and refilled with nitrogen three times. The temperature was then brought to reflux for about 30 min until a yellow precipitate had formed. The mixture was filtered and the solids washed with hexane to give pure dimer **33**. Compound **33**: mp >350 °C (dec) IR(KBr) 3424, 1708, 1627, 1466, 1369, 1302, 1251, 1154, 1107, 1025, 846, 645 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO) $\delta$ 1.63 (s, 9H), 7.60–7.69 (m, 2H), 8.40 (s, 1H), 8.96 (s, 1H), 9.04 (s, 1H), 12.5 (s, 1H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 28.3, 78.8, 82.5, 112.9, 118.1, 121.0, 122.1, 129.5, 131.5, 134.8, 141.1, 163.0,

131.9, 134.2, 138.0, 140.8, 164.9; MS (FAB) 583 (M$^+$, 100). This material was pure by TLC (silica gel).

The synthesis of the ligands in Table 2b had been reported previously in Ref. 89.

## Acknowledgments

## References and notes

1. Cook, J. M.; Van Linn, M. L.; Yin, W., 2009, 92 pp. CODEN: PIXXD2. (UWM Research Foundation, Inc., USA). WO 2009-US45014 WO 2009143445 A1 CAN 151:571093 AN 2009:1468879.
2. Harvey, S. C.; Foster, K. L.; McKay, P. F.; Carroll, M. R.; Seyoum, R.; Woods, J. E.; Grey, C.; Jones, C. M.; McCane, S.; Cummings, R.; Mason, D.; Ma, C. R.; Cook, J. M.; June, H. L. *J. Neurosci.* **2002**, *22*, 3765.
3. June, H. L.; Foster, K. L.; McKay, P. F.; Seyoum, R.; Woods, J. E.; Harvey, S. C.; Eiler, W. J. A.; Grey, C.; Carroll, M. R.; McCane, S.; Jones, C. M.; Yin, W. Y.; Mason, D.; Cummings, R.; Garcia, M.; Ma, C. R.; Sarma, P.; Cook, J. M.; Skolnick, P. *Neuropsychopharmacology* **2003**, *28*, 2124.
4. Kessler, R. C.; Frank, R. G. *Psychiatr. Med.* **1997**, *27*, 861.
5. O'Malley, S. S.; Jaffe, A. J.; Chang, G.; Schottenfeld, R. S.; Meyer, R. E.; Rounsaville, B. *Arch. Gen. Psychiatry* **1992**, *49*, 881.
6. Volpicelli, J. R.; Alterman, A. I.; Hayashida, M.; O'Brien, C. P. *Arch. Gen. Psychiatry* **1992**, *49*, 876.
7. Kranzler, H. R. *Alcohol* **2000**, *35*, 537.
8. Spanagel, R.; Zieglgansberger, W. *Trends Pharmacol. Sci.* **1997**, 18.
9. Cloninger, C. R. *Science* **1987**, *236*, 410.
10. Li, T.-K. *J. Stud. Alcohol* **2000**, *61*, 5.
11. Li, T.-K.; Crabb, D. W.; Lumeng, L. *Neuropharmacology of Ethanol*; Birkhauser: Boston, 1991. pp 107–124.
12. Johnson, B. A.; Ait-Daoud, N. *Psychopharmacology* **2000**, *149*, 327.
13. Koob, G. F.; Roberts, A. J.; Schulteis, G. et al. *Alcohol Clin. Exp. Res.* **1998**, *22*, 3.
14. June, H. L.; Cason, C. R.; Cheatham, G.; Liu, R. Y.; Gan, T.; Cook, J. M. *Brain Res.* **1998**, *794*, 103.
15. McBride, W. J.; Li, T.-K. *Crit. Rev. Neurobiol.* **1998**, *12*, 339.
16. Allain, H.; Belliard, S.; Decertaines, J.; Bentueferrer, D.; Bureau, M.; Lacroix, P. *Dementia* **1993**, *4*, 347.
17. Heimer, L.; Alheid, G. F. *The Basal Forebrain: Anatomy and Function*; Plenum Press: New York, 1991. pp 1–42.
18. Heimer, L.; Zahm, D. S.; Churchill, P.; Kalivas, W. *Neuroscience* **1991**, *41*, 89.
19. Criswell, H. E.; Simson, P. E.; Duncan, G. E.; Mc Cown, T. J.; Herbert, J. S.; Morrow, L. J. *J. Pharmacol. Exp. Ther.* **1993**, *267*, 522.
20. Criswell, H. E.; Simson, P. E.; Knapp, D. J.; Devaud, L. L.; Mc Cown, T. J.; Duncan, G. E. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 525.
21. June, H. L.; Zuccarelli, D.; Torres, L.; Craig, K. S.; DeLong, J.; Allen, A.; Braun, M. R.; Cason, C. R.; Murphy, J. M. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 1006.
22. June, H. L.; Harvey, S. C.; Foster, K. L.; McKay, P. F.; Cummings, R.; Garcia, M.; Mason, D.; Grey, C.; McCane, S.; Williams, L. S.; Johnson, T. B.; He, X. H.; Rock, S.; Cook, J. M. *J. Neurosci.* **2001**, *21*, 2166.
23. Hyytia, P.; Koob, G. F. *Eur. J. Pharmacol.* **1995**, *283*, 151.
24. Nowak, K. L.; McBride, W. J.; Lumeng, L.; Li, T.-K.; Murphy, J. M. *Psychopharmacology* **1998**, *139*, 108.
25. Nauta, H. J.; Smith, G. P.; Faull, R. L. M.; Domesick, V. B. *Neuroscience* **1978**, 3.
26. Zahm, D. S.; Heimer, L. *J. Comp. Neurol.* **1988**, *272*, 516.
27. Groenwegen, H. J.; Berende, H. W. *Neuroscience* **1993**, *57*, 113.
28. Churchill, L.; Kalivas, P. W. *J. Comp. Neurol.* **1994**, *345*, 579.
29. Kuo, H.; Chang, H. T. *J. Comp. Neurol.* **1992**, *321*, 626.
30. Morgenson, G. J.; Nielson, M. *Brain Res. Bull.* **1983**, *11*, 309.
31. Fritschy, J. M.; Mohler, H. *J. Comp. Neurol.* **1995**, *359*, 154.
32. Turner, J. D.; Bodewitz, G.; Thompson, C. L.; Stephenson, F. A. *Anxiolytic Beta Carbolines: From Molecular Biology to the Clinic*; Springer: New York, 1993. pp 29–49.
33. Churchill, L.; Bourdelais, A.; Austin, M. C.; Lolait, S. J.; Mahan, L. C.; O'Carroll, A. M.; Kalivas, P. W. *Synapse* **1991**, *8*, 75.
34. Wisden, W.; Laurie, D. J.; Monyer, H.; Seeburg, P. H. *J. Neurosci.* **1992**, *12*, 1040.
35. Duncan, G. E.; Breese, G. R.; Criswell, H. E.; McCown, T. J.; Herbert, J. S.; Devaud, L. L.; Morrow, A. L. *Neuroscience* **1995**, *64*, 1113.
36. Napier, T. C.; Chrobak, J. J. *Neuroreport* **1992**, *3*, 609.
37. Hubner, C. B.; Koob, G. F. *Eur. J. Pharmacol.* **1995**, *283*, 151.
38. Gong, W.; Justice, J. B. *Prog. Neuropsychohpharmacol. Biol. Psychiatry* **1997**, *21*, 839.
39. Gong, W.; Neill, D. *Brain Res.* **1996**, *707*, 64.
40. Cicero, T. J.. In *Biochemistry and Pharmacology of Ethanol*; Plenum Press: New York, 1979; Vol. 2. pp 533–560.
41. Lumeng, L.; Murphy, J. M.; McBride, W. J.; Li, T.-K. *Genetic Influences on Alcohol Preference in Animals*; Oxford University Press: New York, 1995. pp 165–201.
42. June, H. L.; Eggers, M. W.; Warren-Reese, C.; DeLong, J.; Ricks-Cord, A.; Durr, L. F.; Cason, C. R. *Eur. J. Pharmacol.* **1998**, *350*, 151.
43. June, H. L.; Torres, L.; Cason, C. R.; Hwang, B. H.; Braun, M. R.; Murphy, J. M. *Brain Res.* **1998**, *784*, 256.
44. Sieghart, W.; Ernst, M. *Curr. Med. Chem.: Cent. Nerv. Syst. Agents* **2005**, *5*, 217.
45. Bowser, D. N.; Wagner, D. A.; Czajkowski, C.; Cromer, B. A.; Parker, M. W.; Wallace, R. H.; Harkin, L. A.; Mulley, J. C.; Marini, C.; Berkovic, S. F.; Williams, D. A.; Jones, M. V.; Petrou, S. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15170.
46. Bateson, A. N. *Sleep Med.* **2004**, *5*, S9–S15.
47. Otani, K.; Ujike, H.; Tanaka, Y.; Morita, Y.; Katsu, T.; Nomura, A.; Uchida, N.; Hamamura, T.; Fujiwara, Y.; Kuroda, S. *Neurosci. Lett.* **2005**, *381*, 108.
48. Dean, B.; Scarr, E.; McLeod, M. *Brain Res. Mol. Brain Res.* **2005**, *138*, 145.
49. Guidotti, A.; Auta, J.; Davis, J. M.; Dong, E. B.; Grayson, D. R.; Veldic, M.; Zhang, X. Q.; Costa, E. *Psychopharmacology* **2005**, *180*, 191.
50. Maubach, K. *Drug Targets CNS Neurol. Disord.* **2003**, *2*, 233.
51. Barrett, A. C.; Negus, S. S.; Mello, N. K.; Caine, S. B. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 858.
52. Anthenelli, R.; Schuckit, M. A. *Inter. J. Addict.* **1990/1991**, *25*, 81.
53. Schuckit, M. A. *Alcohol Clin. Exp. Res.* **1988**, *12*, 465.
54. Barnard, E.; Skolnick, P.; Olsen, R.; Möhler, H.; Sieghart, W.; Biggio, G.; Braestrup, C.; Bateson, A.; Langer, S. *J. Pharmacol. Exp. Ther. (Pharmacol. Rev.)* **1998**, *50*, 291.
55. Simon, J.; Wakimoto, H.; Fujita, N.; Lalande, M.; Barnard, E. A. *J. Biol. Chem.* **2004**, *279*, 41422.
56. Sieghart, W.; Sperk, G. *Curr. Top. Med. Chem.* **2002**, *2*, 795.
57. Mossier, B.; Togel, M.; Fuchs, K.; Sieghart, W. *J. Biol. Chem.* **1994**, *269*, 25777.
58. Togel, M.; Mossier, B.; Fuchs, K.; Sieghart, W. *J. Biol. Chem.* **1994**, *269*, 12993.
59. Pirker, S.; Schwarzer, C.; Wieselthaler, A.; Sieghart, W.; Sperk, G. *Neuroscience* **2000**, *101*, 815.
60. (a) Cox, E. D.; Diaz-Arauzo, H.; Huang, Q.; Reddy, M. S.; Harris, B.; Mckernan, R. M.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1998**, *41*, 2537; (b) Huang, Q. Ph.D. Thesis, University of Wisconsin-Milwaukee, 1998.
61. Huang, H.; He, X.; Ma, C. R.; Liu, R. Y.; Yu, S.; Dayer, C. A.; Wenger, G. R.; McKernan, R.; Cook, J. M. *J. Med. Chem.* **2000**, *43*, 71.
62. Carroll, M.; Woods, J. E., II; Seyoum, R. A.; June, H. L. *Alcohol Clin. Exp. Res.* **2001**, 25.
63. Rowlett, J. K. Unpublished results, 2010.
64. June, H. L.; Cook, J. M.; Ma, C. U.S. Patent Appl. Publ., 2003, 60 pp. CODEN: USXXCO. (USA). US 2002-329100 US 20030176456 AN 2003:737374 CAN 139:241570.
65. Foster, K. L.; McKay, P. F.; Seyoum, R.; Milbourne, D.; Yin, W.; Sarma, P. V. V. S.; Cook, J. M.; June, H. L. *Neuropsychopharmacology* **2004**, *29*, 269.
66. Araki, T.; Tohyama, M. *Mol. Brain Res.* **1992**, *12*, 293.
67. Cox, E. D.; Hagen, T. J.; Mckernan, R. M.; Cook, J. M. *Med. Chem. Res.* **1995**, *5*, 710.
68. Griebel, G.; Perrault, G.; Letang, V.; Grainger, P.; Avenet, P.; Schoemaker, H. *Psychopharmacology* **1999**, *146*, 205.
69. Carroll, M. E.; Carmona, G.; May, S. *J. Exp. Anal. Behav.* **1991**, *18*, 361.
70. Paronis, C. A.; Cox, E. D.; Cook, J. M.; Bergman, J. *Psychopharmacology* **2001**, *156*, 461.
71. Rowlett, J. K.; Tornatzky, W.; Cook, J. M.; Ma, C. R.; Miczek, K. A. *J. Pharmacol. Exp. Ther.* **2001**, *297*, 247.
72. Shannon, H. E.; Guzman, F.; Cook, J. M. *Life Sci.* **1984**, *35*, 2227.
73. Rowlett, J. K.; Spealman, R. D.; Lelas, S.; Cook, J. M.; Yin, W. Y. *Psychopharmacology* **2003**, *165*, 209.
74. Rowlett, J. K.; Cook, J. M.; Duke, A. N.; Platt, D. M. *CNS Spectr.* **2005**, *10*, 40.
75. Liu, R.; Zhang, P.; Mckernan, R. M.; Wafford, K. A.; Cook, J. M. *Med. Chem. Res.* **1995**, *5*, 700.
76. Liu, R.; Hu, R. J.; Zhang, P.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1996**, *39*, 1928.
77. Li, X. Y.; Cao, H.; Zhang, C. C.; Furtmueller, R.; Fuchs, K.; Huck, S.; Sieghart, W.; Deschamps, J.; Cook, J. M. *J. Med. Chem.* **2003**, *46*, 5567.
78. Allen, M. S.; Tan, Y. C.; Trudell, M. L.; Narayanan, K.; Schindler, L.; Martin, M. J.; Schultz, C. A.; Hagen, T. J.; Koehler, K. F.; Codding, P.; Skolnick, P.; Cook, J. *J. Med. Chem.* **1990**, *33*, 2343.
79. Allen, M. S.; Laloggia, A. J.; Dorn, L. J.; Martin, M. J.; Constantino, G.; Hagen, T. J.; Koehler, K. F.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1992**, *35*, 4001.
80. Cain, M.; Weber, R. W.; Guzman, F.; Cook, J. M.; Barker, S. A.; Rice, K. C.; Crawley, J. N.; Paul, S. M.; Skolnick, P. *J. Med. Chem.* **1982**, *25*, 1081.
81. Hagen, T. J.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1987**, *30*, 750.
82. Cook, J. M.; Diaz-Arauzo, H.; Allen, M. S. *NIDA Res. Monogr.* **1990**, 133.
83. Schweri, M.; Cain, M.; Cook, J. M.; Paul, S.; Skolnick, P. *Pharmacol. Biochem. Behav.* **1982**, *17*, 457.
84. Fryer, R. I.; Cook, C.; Gilman, N. W.; Walser, A. *Life Sci.* **1986**, *39*, 1947.
85. Trullas, R.; Ginter, H.; Jackson, B.; Skolnick, P.; Allen, M. S.; Hagen, T. J.; Cook, J. M. *Life Sci.* **1988**, *43*, 1193.
86. Ninan, P. T.; Insel, T. M.; Cohen, R. M.; Cook, J. M.; Skolnick, P.; Paul, S. M. *Science* **1982**, *218*, 1332.
87. Mendelson, W. B.; Cain, M.; Cook, J. M.; Paul, S. M.; Skolnick, P. *Science* **1983**, *219*, 414.
88. Hagen, T. J.; Guzman, F.; Schultz, C.; Cook, J. M.; Shannon, H. E. *Heterocycles* **1986**, *24*, 2845.
89. Allen, M. S.; Hagen, T. J.; Trudell, M. L.; Codding, P. W.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1988**, *31*, 1854.

90. Diaz-Arauzo, H.; Evoniuk, G. E.; Skolnick, P.; Cook, J. M. *J. Med. Chem.* **1991**, *34*, 1754.
91. Wafford, K. A.; Bain, C. J.; Whiting, P. J.; Kemp, J. A. *Mol. Pharmacol.* **1993**, *44*, 437.
92. Braestrup, C.; Nielsen, M. *Nature* **1981**, *294*, 472.
93. Venault, P.; Chapouthier, G.; de-Carvalho, L. P.; Simiand, J.; Morre, M.; Dodd, R. H.; Rossier, J. *Nature* **1986**, *321*, 864.
94. Lippke, K. P.; Schunack, W. G.; Wenning, W.; Müller, W. E. *J. Med. Chem.* **1983**, *26*, 499.
95. Corda, M. G.; Blaker, W. D.; Mendelson, W. B.; Guidotti, A.; Costa, E. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2072.
96. Havoundjian, H.; Reed, G. F.; Paul, S. M.; Skolnick, P. *J. Clin. Invest.* **1987**, *79*, 473.
97. Hadingham, K. L.; Wingrove, P.; Le-Bourdelles, B.; Palmer, K. J.; Ragan, C. I.; Whiting, P. J. *Mol. Pharmacol.* **1993**, *43*, 970.
98. Sanger, D. J.; Benavides, J.; Perrault, G.; Morel, E.; Cohen, C.; Joly, D.; Zivkovic, B. *Neurosci. Biobehav. Rev.* **1994**, *18*, 355.
99. McKernan, R. M.; Rosahl, T. W.; Reynolds, D. S.; Sur, C.; Wafford, K. A.; Atack, J. R.; Farrar, J.; Cook, G.; Ferris, P.; Garrett, L.; Bristow, L.; Marshall, G.; Macaulay, A.; Brown, N.; Howell, O.; Moore, K. W.; Carling, R. W.; Street, L. J.; Castro, J. L.; Ragan, C. I.; Dawson, G. R.; Whiting, P. J. *Nat. Neurosci.* **2000**, *3*, 587.
100. Braestrup, C.; Nielsen, M.; Olsen, R. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2288.
101. Nielsen, M.; Braestrup, C. *Nature* **1980**, *286*, 606.
102. Huang, Q.; Cox, E. D.; Gan, T.; Ma, C. R.; Bennett, D. W.; Mckernan, R. M.; Cook, J. M. *Drug. Des. Discov.* **1999**, *16*, 55.
103. Yin, W.; Sarma, P. V. V. S.; Ma, J.; Han, D.; Chen, J. L.; Cook, J. M. *Tetrahedron Lett.* **2005**, *46*, 6363.
104. Portoghese, P. S. *J. Med. Chem.* **2001**, *44*, 2259.
105. Portoghese, P. S.; Lin, C. E.; Farouzgrant, F.; Takemori, A. E. *J. Med. Chem.* **1994**, *37*, 1495.
106. Halazy, S. *Exp. Opin. Ther. Patents* **1999**, *9*, 431.
107. June, H. L., Sr.; Foster, K. L.; Eiler, W. J. A. I.; Goergen, J.; Cook, J. B.; Johnson, N.; Mensah-Zoe, B.; Simmons, J. O.; June, H. L., Jr.; Yin, W.; Cook, J. M.; Homanics, G. E. *Neuropsychopharmacology* **2007**, *32*, 137.
108. Bell, R. L.; Rodd, Z. A.; Lumeng, L.; Murphy, J. M.; McBride, W. J. *Addict. Biol.* **2006**, *11*, 270.
109. Koob, G. F. *Biochem. Pharmacol.* **2004**, *68*, 1515. review and references cited therein.
110. Clayton, T.; Chen, J. L.; Ernst, M.; Richter, L.; Cromer, B. A.; Morton, H. N.; Kaczorowski, C. C.; Helmstetter, F. J.; Furtmuller, R.; Ecker, G.; Parker, M. W.; Sieghart, W.; Cook, J. M. *Curr. Med. Chem.* **2007**, *14*, 2755.
111. Cromer, B.; Morton, C.; Parker, M. W. *Trends Biochem. Sci.* **2002**, *27*, 280.
112. Trudell, J. R. *Biochim. Biophys. Acta* **2002**, *1565*, 91.
113. Ma, C. Ph.D. Thesis, University of Wisconsin-Milwaukee, 2000.
114. Ramachandran, P. V.; Teodorovic, A. V.; Brown, H. C. *Tetrahedron* **1993**, *49*, 1725.
115. Ramachandran, P. V.; Gong, B.; Brown, H. C. *J. Org. Chem.* **1995**, *60*, 41.
116. Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, *50*, 4467.
117. Sonogashira, K. *Organometal. Chem.* **2002**, *653*, 46.
118. Lei, A.; Srivastava, M.; Zhang, X. *J. Org. Chem.* **2002**, *67*, 1969.
119. Choudhary, M. S.; Craigo, S.; Roth, B. L. *Mol. Pharmacol.* **1992**, *42*, 627.
120. Lüddens, H.; Korpi, E. R.; Seeburg, P. H. *Neuropharmacology* **1995**, *34*, 245. Review.
121. Huang, Q.; Zhang, W. J.; Liu, R. Y.; McKernan, R. M.; Cook, J. M. *Med. Chem. Res.* **1996**, *6*, 384.
122. Huang, Q.; Liu, R. Y.; Zhang, P. W.; He, X. H.; McKernan, R.; Gan, T.; Bennett, D. W.; Cook, J. M. *J. Med. Chem.* **1998**, *41*, 4130.
123. He, X. H.; Huang, Q.; Ma, C. R.; Yu, S.; McKernan, R.; Cook, J. *Drug Des. Discov.* **2000**, *17*, 131.
124. O'Mara, M.; Cromer, B.; Parker, M.; Chung, S. H. *Biophys. J.* **2005**, *88*, 3286.
125. Arnold, K.; Bordoli, L.; Kopp, J.; Schwede, T. *Bioinformatics* **2006**, *22*, 195.
126. Buhr, A.; Baur, R.; Sigel, E. *J. Biol. Chem.* **1997**, *272*, 11799.
127. Buhr, A.; Schaerer, M. T.; Baur, R.; Sigel, E. *Mol. Pharmacol.* **1997**, *52*, 676.
128. Buhr, A.; Sigel, E. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8824.
129. Mihic, S. J.; Whiting, P.; Klein, R. L.; Wafford, K.; Harris, R. A. *J. Biol. Chem.* **1994**, *269*, 32768.
130. Sigel, E.; Schaerer, M. T.; Buhr, A.; Baur, R. *Mol. Pharmacol.* **1998**, *54*, 1097.
131. Braestrup, C.; Honore, T.; Nielsen, M. C.; Peterson, E. N.; Jensen, L. H. *Biochem. Pharmacol.* **1984**, *33*, 859.
132. Lawson, J.; Uyeno, E. T.; Nienow, J.; Loew, G. H.; Toll, L. *Life Sci.* **1984**, *34*, 2007.
133. Hanzawa, Y.; Kawagoe, K.; Ito, M.; Kobayashi, Y. *Chem. Pharm. Bull.* **1987**, *35*, 1633.
134. Liu, R.; Zhang, P.; Gan, T.; Mckernan, R. M.; Cook, J. M. *Med. Chem. Res.* **1997**, *7*, 25.
135. Saiga, Y.; Iijima, I.; Ishida, A.; Miyagishima, T.; Shigezane, K.; Oh-Ishi, T.; Matsumoto, M.; Matsuoka, Y. *Chem. Pharm. Bull.* **1987**, *35*, 3262.
136. Eftink, M. R.; Jia, J.; Hu, D.; Ghiron, C. A. *J. Phys. Chem.* **1995**, *99*, 5713.
137. Plate, R.; Nivard, R. J. F.; Ottenheijm, H. C. J.; Kardos, J.; Simonyi, M. *Heterocycles* **1986**, *24*, 3105.
138. Moody, C. J.; Ward, J. G. *J. Chem. Soc. Perkin Trans. 1* **1984**, 2895.