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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[1], **Samarpan Majumder**[2], **Terry Clayton**[1], **Steven Petrou**[3], **Michael L. VanLinn**[1], **Ojas A. Namjoshi**, **Chunrong Ma**[1], **Brett A. Cromer**[3,4], **Bryan L. Roth**[2], **Donna M. Platt**[5], and **James M. Cook**[1,*]

[1] Department of Chemistry and Biochemistry, University of Wisconsin-Milwaukee, Milwaukee, WI, 53201-0413

[2] 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

[3] Howard Florey Institute, University of Melbourne, Parkville, VIC 3010, Australia

[4] Department of Pharmacology, University of Melbourne, Parkville, VIC 3010, Australia

[5] Harvard Medical School, New England Primate Research Center, One Pine Hill Drive, Box 9102, Southborough, MA 01772-9102

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 α_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*) α_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

*capncook@uwm.edu.

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effect on sucrose self-administration.[1–3] These data prompted the synthesis of the β-carbolines presented here.

Introduction

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 alcoholism,[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 receptors containing the α1 receptor subtype (GABA α1) appear preeminent. Thus, Criswell and colleagues observed that acute alcohol administration selectively enhanced the effects of ionotophoretically 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 caudate.[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

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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 5 subunits that form a central chloride channel and can belong to different subunit classes. A total of 19 subunits (6α, 3β, 3γ, 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 the $GABA_A$ receptors in alcohol reinforcement and better understand neurobehavior and ethanol responding.

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. Behavioural studies in several species (eg, rats, mice, primates) show that βCCt is a BDZ antagonist exhibiting competitive binding site interaction 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 anticonflict 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 α_1 subtype selectivity.[67,74–77] The discovery of high affinity, saturable, and

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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 5 recombinant $GABA_A$/BzR subtypes ($\alpha_1\beta_3\gamma_2$, $\alpha_2\beta_3\gamma_2$, $\alpha_3\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$ and $\alpha_6\beta_3\gamma_2$) expressed from recombinant human cell lines.[91,97] In general, this series of β-carboline ligands exhibited some selectivity at α_1 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 α_1 subtype over the α_2 and α_3 receptors, as well as over 150-fold selectivity for the α_1 site over the α_5 subtype.[2,3] βCCt (**1**) was more selective at the α_1 subtype *in vitro* than the classical α_1 selective agonists zolpidem (**3**) and CL 218872 (**4**) (Figure 1).[60,98,99] A number of *in vitro* and *in vivo* studies employing α_1 (e.g., zolpidem, CL 218872,[68] βCCt, and 3-PBC[3]) selective ligands suggest the α_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]

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 α_1 containing receptor isoform and this was confirmed by *in vitro* binding affinity of these ligands.[102] A lipophilic region (L_{Di}) of the pharmacophore receptor model appears to be larger in the α_1, α_2 and α_3-containing receptor isoforms and important for α_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 Figure 1), including 6-iodo-βCCt (**5**) and 6-trimethylsilanyl-ethynyl-βCCt (**6**) (Figure 1) possessed α_1 subtype selectivity.[103]

The rigidly linked linear bivalent ligands of βCCt at position "**6**" did bind to BzR receptors with some α_1 subtype selectivity and may provide the desired α_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 α_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 nonselective antagonist flumazenil (Ro 15-1788, for which intrinsic efficacy at all BZ-sensitive $GABA_A$ subtype was relatively low, but not zero). To date, no compound has been characterized that exhibits zero efficacy at all

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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 was indeed critical to the knowledge of their mode of action in the CNS.

Based on the limited availability of data on the series of α_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 α_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 α_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]

Chemistry

The synthesis of the ligands under study is outlined in Schemes 1, 2 and 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-Spengler reaction on kilogram scale. Fischer esterification of **11**, followed by oxidation with activated MnO_2 provided the intermediate BCCE (**8**) on 200 gram 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 (**1**) 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 gram levels to furnish 3PBC hydrochloride salt in reasonable yield for studies in primates.

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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 α_1 chiral selective analogs CMD-30 R/S isomers (**21** & **22**) can be synthesized by the CDI method in 90% yield (individually) on 10 gram 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_3COOAg 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.

Results and Discussion

(1) βCCt bivalent ligands

The *in vitro* biological protocols employed in the present study follow the published procedure[119,120] and are detailed in the Experimental Section. Although the $\alpha_1\beta_3\gamma_2$ BzR/GABAergic subtype is very similar in structure to the α_2 and α_3 subtypes, there are slight differences.[61,121] One major difference is in region L_{Di}, which appears larger in the α_1 subtype than in either the α_2, or α_3 or α_5 subtypes. This is located near position -6 of βCCt (**1**) and can be seen in the model of the Comparative Molecular Field Analysis (CoMFA) study for the α_1 subtype (Figure 3).[110,122] In particular, blue contours in the western region of the pharmacophore/receptor model imply 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 α_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 α_1-

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preferring ligands focused on design and synthesis of βCCt bivalent ligands. Although the α_1 subtype selectivity was not amplified with the particular acetylenyl linked bivalent ligand **32**, the ligand does bind preferentially at α_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 α_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 (Figure 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 α_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.

(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 α_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 α_1 subtype. However, the most selective ligand for the α_1 subtype was WYS8 (**7**). This α_1 subtype selective ligand was 100 fold more selective over the other subtypes. This was the most α_1 subtype selective ligand reported, to date, to these authors' knowledge. This 6-substituted acetylenyl βCCt **7** was 214 fold more selective for α_1 isoforms over α_5 isoforms. Studies of SAR in Table 1 confirmed the occupation of region L_{Di} of the receptor pharmacophore model did enhance α_1 selectivity in comparison to the affinity of the non-selective ligand diazepam or the α_5 selective ligand, RY080. As illustrated in the two dimensional Figure 5, full occupation of the L_{Di} lip β-carbolines may account for the potency/selectivity of this class of ligands at the α_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 α_1 receptor subtype. In addition, it is important to note that binding affinity in this series of ligands of greater than 400nM 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.

(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 substituent which interacted at L_1. The L1 pocket tolerates linear groups

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up to 4 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 α_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.

(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 4-fold 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 lipophilc 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 α_1 subtype over the α_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 α_1 subtype selective ligands. In fact, they did not bind to BzR at all in agreement with previous work.[123]

(5) Efficacy of α_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 α_1, α_2, α_3, and α_5 receptor subunits in the *Xenopus* oocytes assay and is depicted in Figure 2 by 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 α_1 (96±7%), α_2 (99±10%), α_3 (108±6%), and α_4 (107±5%) receptors. However, a low-efficacy partial inverse agonist response was observed at the α_5 receptor (88±7% of the GABA response). Flumazenil exhibited an efficacy profile that was qualitatively similar to βCCt at the α_1 (99±5%), α_3 (118±7%), and α_5 (96±6%) subtypes. At the α_2 receptor, flumazenil produced a low-efficacy agonist response

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(115±4%), while βCCt was GABA neutral (99±10%). Flumazenil also produced a qualitatively similar response to βCCt at the α_4 receptor, albeit the magnitude of GABA potentiation by flumazenil far exceeded that of βCCt (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 α_1–α_4 subtypes, but was GABA neutral at the α_5 receptor (96±6%). These findings suggested that βCCt 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, βCCt and 3-PβC 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 βCCt, which targeted the $GABA_A$ α_1 receptor as a weak agonist-like antagonist,[74] should facilitate efforts to understand the antialcohol action of β-carbolines in nonhuman primates and humans as well.

In the NIMH supported PDSP screen neither βCCT, 3PBC, nor WYS8 exhibited significant interactions at other receptors (see http://pdsp.med.unc.edu for details).

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 β-carboline ligands described here has exhibited some selectivity at the α_1 receptor subtype which included β-carboline-3-carboxylate-*t*-butyl ester (βCCt, **1**) and 3-propoxy-β-carboline hydrochloride (3-PBC, **2**). These ligands displayed a 20-fold and 10-fold selectivity, respectively, for the α_1 subtype over the α_2 and α_3 receptors, as well as over 150-fold selectivity for the α_1 site over the α_5 subtype.[2,3] βCCt (**1**) was more selective at the α_1 subtype *in vitro* than the classical α_1 selective agonists zolpidem (**3**) and CL 218872 (**4**).[60,98,99] βCCt and 3-PBC are capable of competitive antagonism of classical benzodiazepine agonists,[3,60,72] therefore, the development of subtype-selective antagonists for $GABA_A$ receptors which targets the $GABA_A$ α_1 receptor as a weak agonist-like antagonist,[74] should facilitate efforts to understand the antialcohol 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 α_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 α_1 selectivity in comparison to the affinity of the non-selective ligands or the α_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 α_1 subunit. Based on the SAR, the most potent α_1 selective ligand was 6-substituted acetylenyl βCCt (WYS8, **7**). It was suggested the attenuation of EtOH-motivated responding effected by WYS8

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(**7**) was mediated *via* the α_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 4-fold selectivity for the $\alpha_1\beta_3\gamma_2$ subtype, the (R) isomer remained more potent *in vitro* at all 5 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 α_1 subtypes, to date, to these authors' knowledge was WYS8 (**7**). This 6-substituted acetylenyl βCCt (**7**) was 214 fold more selective for α_1 isoforms over α_5 isoforms. WYS8 can clearly be differentiated from nonselective BDZs by its selective binding affinity at the α_1 receptor subunit and reduced capacity to potentiate GABA in *Xenopus* oocytes.

Innate elevations of the α_1 and α_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 α_{1-2} subunits in potentiating GABA may render it a safe BDZ receptor ligand devoid of synergistic interactions with alcohol.

Experimental Section

Biological & 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 hour 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 degree) 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 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

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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.

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** (953g, 90.0%). **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.

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 12L (3 neck) flask, and conc 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_2O (6.4 L) and the pH of the solution adjusted to 8 with cold aq NH_4OH (conc.) after which a precipitate formed. This mixture was then extracted with $CHCl_3$ (6 × 2.5 L). The combined organic layers were dried (Na_2SO_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%). **11:** mp 150 ° C (lit. mp 149–150 °C);[90,137] ^1H (300 MHz, $CDCl_3$) δ 1.33 (t, *J* = 7.32 Hz, 3H), 2.45 (s(br), 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), 4.07 (s(br), 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 (s(br), 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.

Ethyl 9H-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** (200g, 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 (silica 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 (100–120g, 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. **8:** mp 225–227 °C (lit. 224–229 °C);[137,138] ^1H (300 MHz, DMSO-d_6) δ 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.

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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%, 1.5L) 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 conc HCl to pH 4. The precipitate which resulted was stirred overnight. The solid was collected by vacuum filtration and washed with H_2O (2 × 150 mL). The product was dried at 80 °C under vacuum for 24 h to provide **9** (26.1 g, 99%). **9:** mp 220–221 °C (lit. mp 220 °C);[67] IR (KBr) 3260, 2970, 1710 cm^{-1}; ^1H NMR ($CDCl_3$) δ 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 $C_{16}H_{16}N_2O2$ (0.55 H_2O): C, 69.07; H, 6.19; N, 10.07. Found: C, 68.81; H, 5.77; N, 10.00.

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 2h 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 eq). The mixture was heated at 85°C for 18h until analysis by TLC indicated the disappearance of the imidazole intermediate. The solvent was then 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}; ^1H (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]

2,2,2-Trifluoroethyl β-carboline-3-carboxylate 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. 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 (s, br, 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.

9H-β-Carboline-3-carboxylic acid 2,2,2-trifluoro-1-trifluoromethyl-ethyl ester 16 was prepared following the procedure employed for the preparation of **1. 16:** ^1H 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).

9H-β-Carboline-3-carboxylic acid 2,2,2-trichloro-ethyl ester 17 was prepared following the procedure employed for the preparation of **1. 17:** ^1H 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).

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9H-β-Carboline-3-carboxylic acid 2,2,2-trifluoro-1-methyl-ethyl ester 18 was prepared following the procedure employed for the preparation of **1. 18:** m.p. 247 249 °C; IR (NaCl) 2359, 1729, 1345, 1251, 1092, 729, 450 cm^{-1}; ^1H 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).

(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 eq) 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 diaspperance 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. **21**: mp 239–241 °C; $[\alpha]_D^{27} = -9.62^\circ$ (c = 0.81, in $CHCl_3$); IR (NaCl) 3266, 1725, 1502 cm^{-1}; ^1H 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 (s, br, 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.

(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 eq). 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 (2.5M, 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**: ^1H 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.

(R)-1,1,1-Trifluoroisopropyl β-carboline-3-carboxylate 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. **22:** mp 239–241 °C; $[\alpha]_D^{27} = 8.73^\circ$ (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.

(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

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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. **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, $CDCl_3$) δ 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 (s, br, 1H); MS (CI, CH_4) *m/e* (relative intensity) 269 (M^+ + 1, 100), 241(15), 213(41), 195(14). Anal. Calcd. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.33; H, 6.09; N, 10.26.

(R)-β-Carboline-3-carboxylic acid sec-butyl ester 24 was prepared in 75% yield following the procedure for preparation of **1. 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-Iodo-*9H*-β-carboline-3-carboxylic acid t-butyl ester (5)—Into a round bottom flask (250 mL) was added CF_3CO_2Ag (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_3CO_2Ag (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. **5**: mp 347–348 °C (dec.); IR (NaCl) 3223, 1710, 1485, 1323, 1245, 1161, 1104, 1020 $cm^{-1;}$ ^{1}H NMR (300 MHz, $CDCl_3$) δ 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 (s, br, 1H); ^{13}C NMR (75.7 MHz, $CDCl_3$) δ 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 $C_{16}H_{15}IN_2O_2$: C, 48.75; H, 3.84; N, 7.11. Found: C, 49.01; H, 3.91; N, 6.95.

6-Trimethylsilanylethynyl-9H-β-carboline-3-carboxylic acid t-butyl ester (6)— Into a round bottom flask (50 mL) which contained a solution of degassed THF/Et_3N (10 mL/2 mL), was added 6-iodo-*9H*-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 $Pd(PPh_3)_2Cl_2$ and CuI can be used as low as 0.5–1 mol%. The reaction mixture was then degassed 2 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 0.5–1h 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, $CDCl_3$) δ 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, $CDCl_3$) δ 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,

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165.4; EIMS 364(M^+, 38), 293(12), 264(100), 249(48), 124(60). This material was employed directly in the next step.

6-Ethynl-9H-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 eq of TBAF (2.8 mL of 1M 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_2O (10 mL) was added and the mixture was extracted with CH_2Cl_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. **7:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 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-Thiophen-2-yl-9H-β-carboline-3-carboxylic acid t-butyl ester (30a). Representative procedure for preparation of 6-subsitituted β-carbolines—A solution of 6-Iodo-β-carboline-3-carboxylic 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 3 times. The mixture was then heated to 140 °C under nitrogen after which tetrakis (triphenylphosphine) palladium (0) (77 mg, 0.067 mmol, 10mol%) 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_2Cl_2 (3 × 25 mL). The combined extracts were washed with 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 = 5:1) to provide a white solid **30a** (195 mg, 83%). **30a:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.07 (s, 9H), 7.15 (t, 1H), 7.31 (d, *J* = 3Hz, 1H), 7.40 (d, *J* = 3Hz, 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-Furan-2-yl-9H-β-carboline-3-carboxylic acid t-butyl ester 29a was prepared following the procedure for preparation of **30a. 29a:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.63(s, 9H), 6.63 (m, 1H), 6.97 (d, *J* = 6Hz, 1H), 7.70 (d, *J* = 9Hz, 1H), 7.77 (s, 1H), 7.96 (d, *J* = 9Hz, 1H), 8.73 (s, 1H), 8.90 (s, 1H), 8.96 (s, 1H), 12.1 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 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-Furan-2-yl-*9H*-β-carboline-3-carboxylic acid 2,2,2-trifluoro-ethyl ester 29b was prepared following the procedure for preparation of **30a. 29b:** ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.82–4.92 (m, 2H), 6.54 (s, 1H), 6.75 (s 1H), 7.38 (s, 1H), 7.65 (d, *J* = 10Hz, 1H), 7.96 (d, *J* = 10Hz, 1H), 8.5 (s, 1H), 8.93 (s, 1H), 9.13 (s, 1H), 9.59 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 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-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_2Cl_2 (30mL), $(Boc)_2O$ (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. **35:** mp 317–320 °C; IR (NaCl) 2975, 2917, 1728, 1457, 1343, 1238, 1154, 1119, 1031,

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811cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.71 (s, 9H), 1.77 (s, 9H), 7.91 (d, *J* = 8.972 Hz, 1H), 8.22 (d, *J* = 8.972Hz, 1H), 8.42 (s, 1H), 8.61 (s, 1H), 9.61 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 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 $C_{21}H_{23}IN_2O_4$(0.1 C_6H_{14}): C, 51.60; H, 4.85; N, 5.57; Found: C, 51.84; H, 4.88; N: 5.45.

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_2Cl_2 = 7:3) to give **36** (4.36 g, 94%) as a white solid. **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, $CDCl_3$) δ 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, $CDCl_3$) δ 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-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** (2.14g, 4.6 mmol) in THF (20 mL), 1.2 eq of TBAF (5.52 ml of 1M 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_2O (10 mL) was added and the mixture was extracted with CH_2Cl_2 (20 mL, 3X). The combined organic layer was concentrated under reduced pressure and the residue was chromatographed on a short column (silica gel, hexane/CH_2Cl_2 = 4:1) to give **34** (1.66 g, 92%) as a white solid. **34:** mp 226–229 °C; IR (NaCl) 3303, 2978, 2346, 2232, 1734, 1622, 1560, 1463 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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 $C_{23}H_{24}N_2O_4$ (0.05 CH_2Cl_2): C, 69.78; H, 6.12; N, 7.06; Found: C: 69.70; H: 6.10; N: 6.81.

1,2-Bis(9H-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** (1.6g, 4.1mmol) and 6-iodo-β-carboline-3,9-dicarboxylic acid di-tert-butyl ester **35** (2.1g, 4.25mmol) 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 1h 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_2Cl_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, 624cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.72 (s, 9H), 1.79 (s, 9H), 7.86 (d, *J* = 8.97 Hz, 1H), 8.32 (s, 1H), 8.47 (d, *J* = 8.79Hz, 1H), 8.68 (s, 1H), 9.63 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 28.2, 82.1,

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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 $C_{44}H_{46}N_4O_8$ (H_2O): 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** (800mg, 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%). **32:** m.p. >350 °C (dec.); IR (KBr) 3227, 1716, 1327, 1162, 738, 450 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 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 (300MHz, $CDCl_3$) δ 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).

1,4-bis(9H-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 (16mg, 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_2Cl_2) to give **1,4-bis(β-carboline-3,9-dicarboxylic acid di-tert-butyl ester) buta-1,3-diyne** (2.08 g, 65%): ^1H NMR (300 MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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. 33:** mp >350 °C (dec.) IR (KBr) 3424, 1708, 1627, 1466, 1369, 1302, 1251, 1154, 1107, 1025, 846, 645 cm^{-1}; ^1H NMR (300 MHz, DMSO) δ 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, $CDCl_3$) δ 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 reference [89].

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138. Moody CJ, Ward JG. [2,3] Fused indoles. Synthesis of β-carbolines and azepino[4,5-b]indoles from 3-(2-alkylindol-3-yl)-2-azidoacrylates. J Chem Soc Perkin Trans 1984;112:2895–2901.

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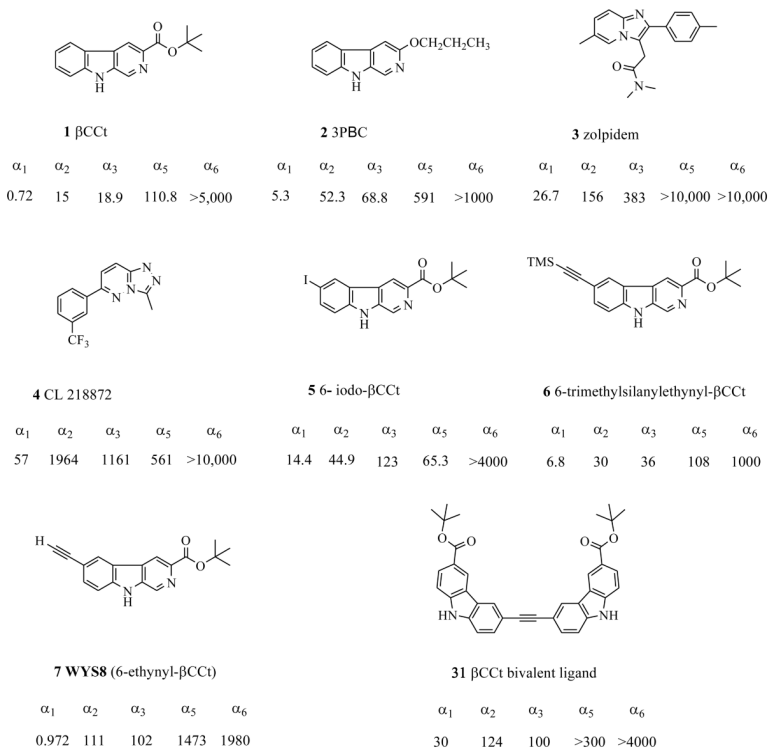

Figure 1.
In vitro binding affinities of a series of α_1 selective ligands (K_i in nM).

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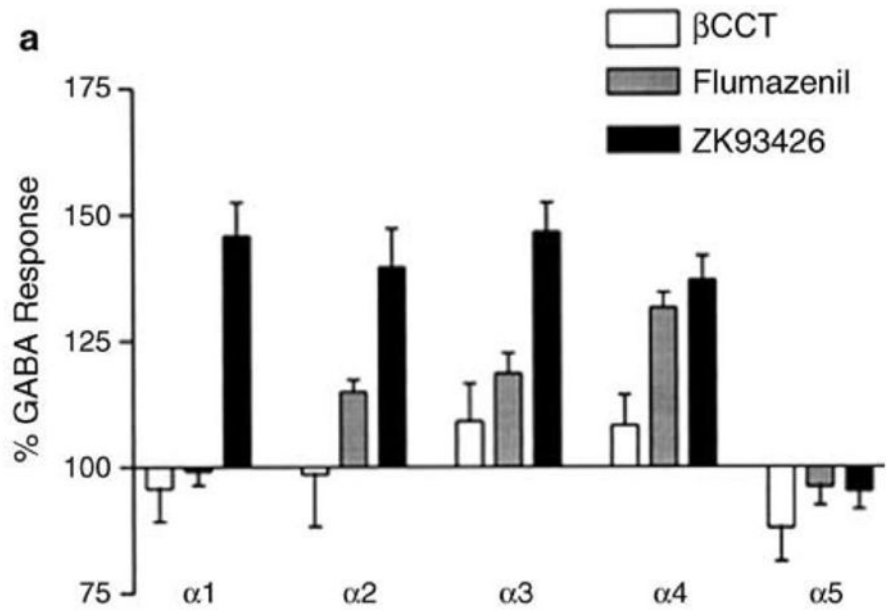

Figure 2.

Efficacy of βCCt in modulating GABA at recombinant $GABA_{\alpha 1\text{-}\alpha 5}$ 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.

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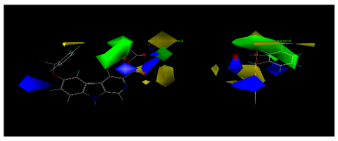

Figure 3.

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.

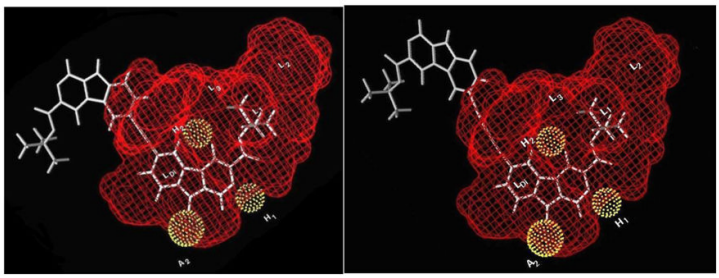

Figure 4.
Alignment of bivalent ligands **32** and **33** within the included volume of the $\alpha_1\beta_3\gamma_2$ subtype.

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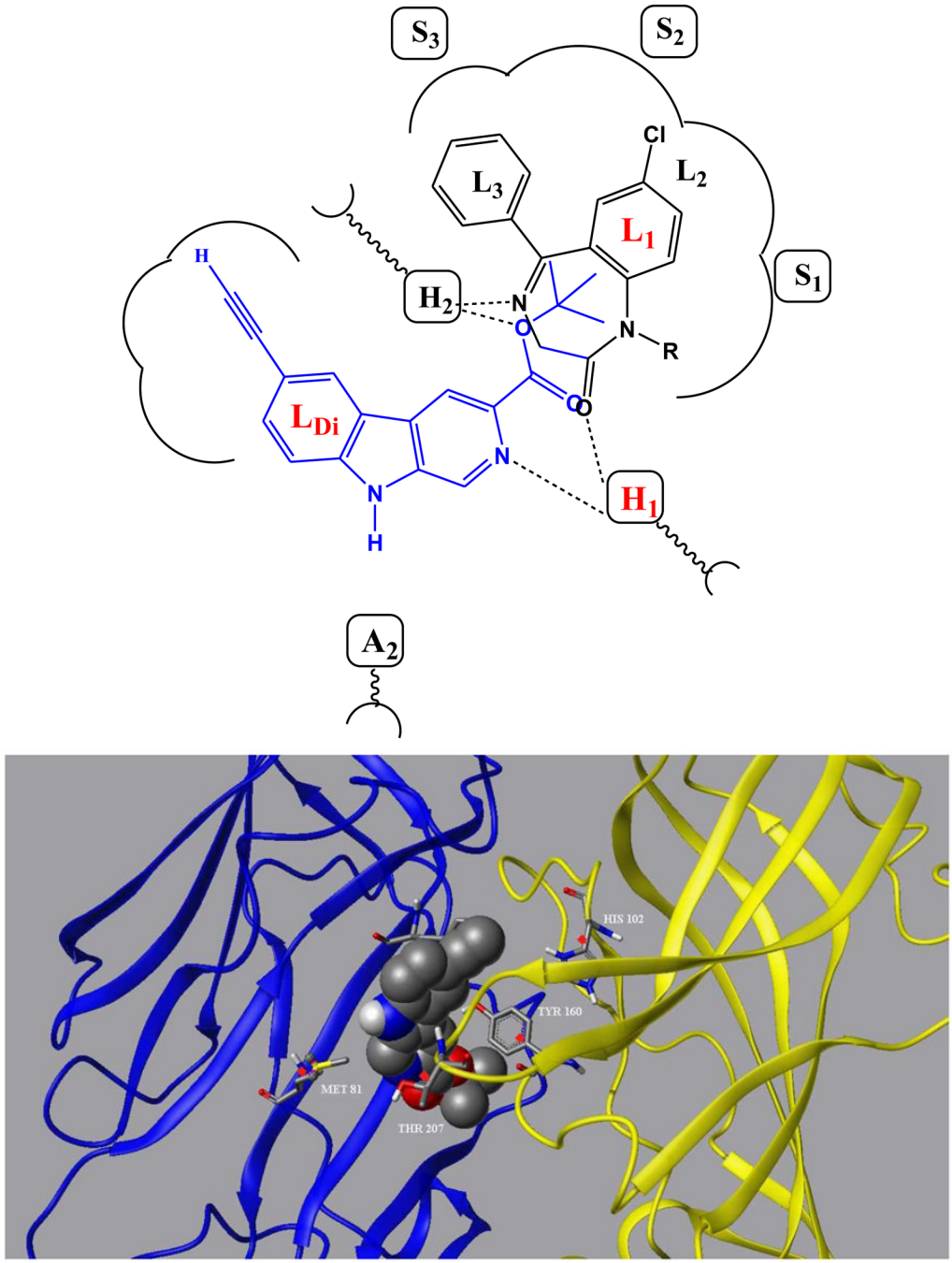
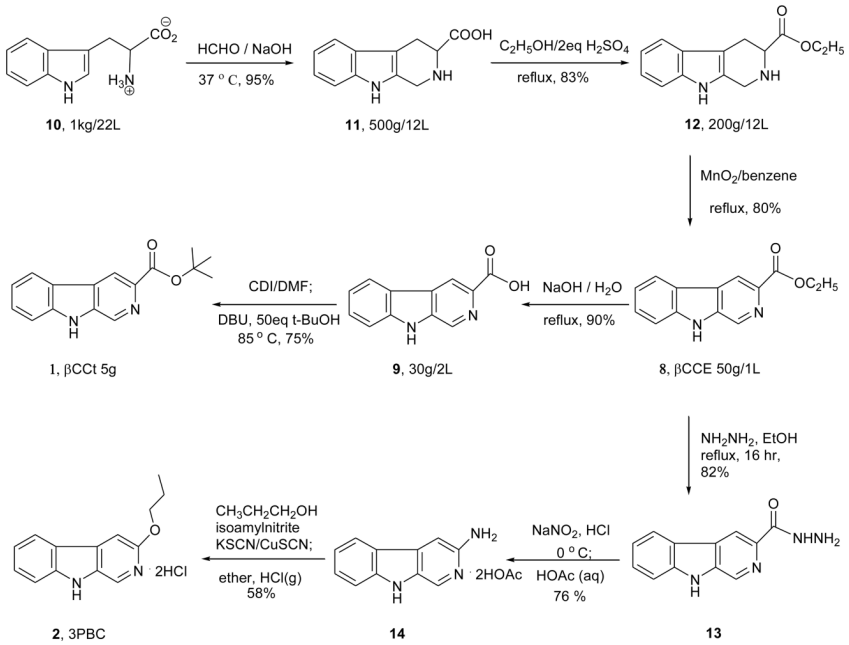


Figure 5.



Scheme 1.

Large scale synthesis of βCCt and synthesis of 3PBC.

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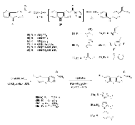

Scheme 2.
CDI-mediated esterification of 3-substituted β-carbolines followed by the conversion into 3,6-disubstituted β-carbolines.

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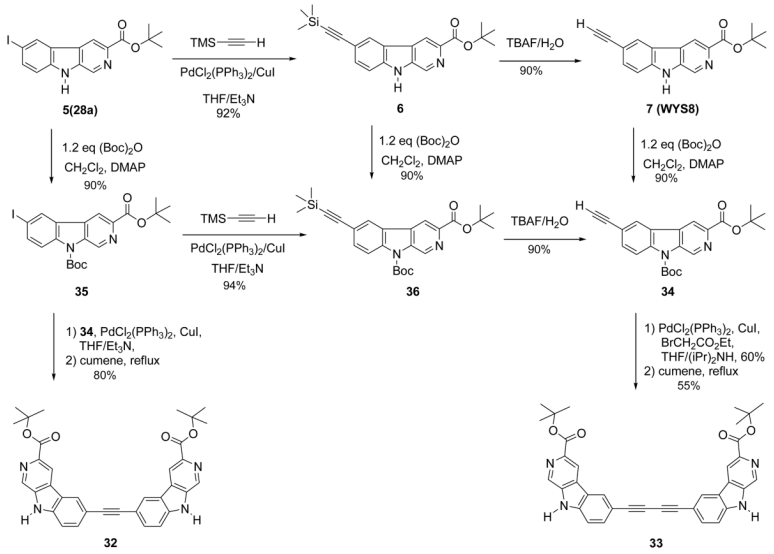

Scheme 3.
Preparation of 6-substituted acetylenyl βCCt (**7, WYS8**) and related bivalent ligands.

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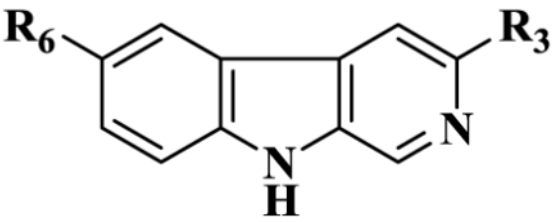
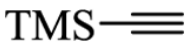
Table 1

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	R_6	R_3	α_1	α_2	α_3	α_4	α_5	α_6
1(βCCt)	**H**	**CO_2tBu**	**0.72**	**15**	**18.9**	**1000**	**111**	**>5,000**
8(βCCE)	**H**	**CO_2Et**	**1.2**	**4.9**	**5.7**	**ND**	**26.8**	**2,700**
2(3-PBC)	H	OnPr	5.3	52.3	68.8	1000	591	>1,000
6(WYB14)		CO_2tBu	6.8	30	36	2000	108	1000
6b(WY-B-25)		$CO_2CH_2CF_3$	17	59	88	200	1444	>3000
6c(WY-B-99-1)		CO_2Et	4.4	4.5	5.58	2000	47	2000
7(WYS8)		CO_2tBu	**0.972**	**111**	**102**	**2000**	**1473**	**1980**

Ligands	R_6	R_3	α_1	α_2	α_3	α_4	α_5	α_6
7b(WY-B-26-2)		$CO_2CH_2CF_3$	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

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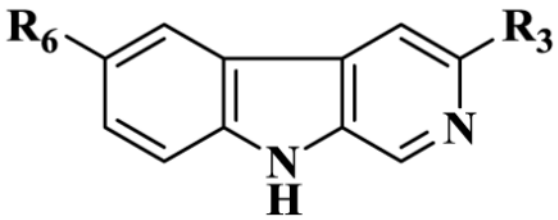
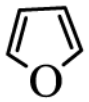
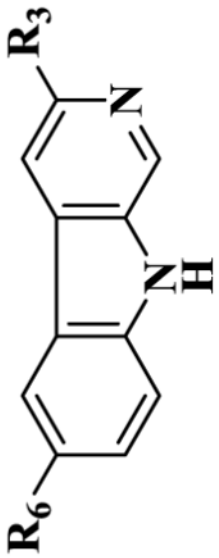


Ligands	R_6	R_3	α_1	α_2	α_3	α_4	α_5	α_6
29a(WYS13)		CO_2tBu	2.4	13	27.5	NA	163	5000
29b(WYB27-1)		$CO_2CH_2CF_3$	26	143	117	3000	127	2000



Ligands	R_6	R_3	α_1	α_2	α_3	α_4	α_5	α_6
30a(WYS12)		CO_2tBu	37	166	314	NA	2861	5000

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Ligands	R_6	R_3	α_1	α_2	α_3	α_4	α_5	α_6
30b(WYB27-2)		$CO_2CH_2CF_3$	9.2	13	72	2000	449	2000

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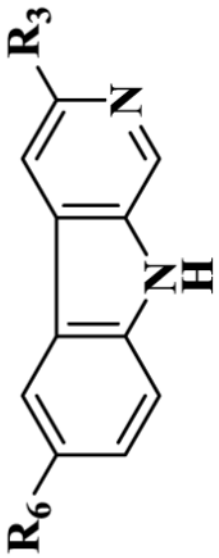

Ligands	R_6	R_3	α_1	α_2	α_3	α_4	α_5	α_6
31a(WYS15)		CO_2tBu	3.63	2.02	44.3	NA	76.5	5000

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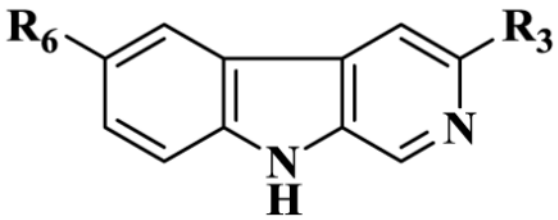
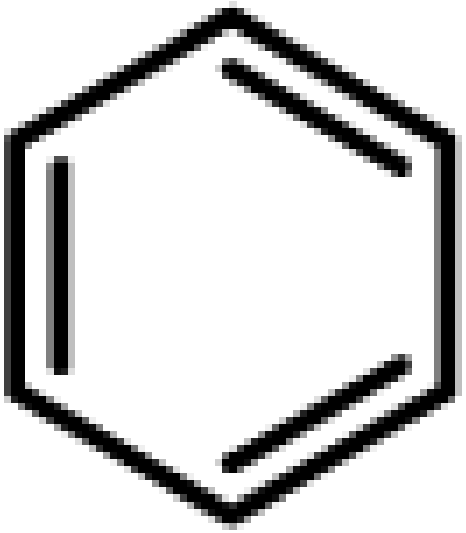
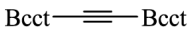


Ligands	R_6	R_3	α_1	α_2	α_3	α_4	α_5	α_6
31b(WYB29-2)		$CO_2CH_2CF_3$	25	137	125	2000	299	2000
CMA57	F	COC_3H_7	3.7	27	40	NA	254	>2500
CM-A-82a	$C(CH_3)_3$	CO_2tBu	2.78	8.93	24.5	1000	7.49	1000
CM-A-87	F	CO_2tBu	1.62	4.54	14.7	1000	4.61	1000
32(WYS2)			30	124	100	>300	>300	>4000
33(WYS6)			120	1059	3942	5000	5000	5000

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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 $\alpha1\beta3\gamma2$, $\alpha2\beta3\gamma2$, $\alpha3\beta3\gamma2$, $\alpha4\beta3\gamma2$, $\alpha5\beta3\gamma2$ and $\alpha6\beta3\gamma2$.[119] Data represent the average of at least three determinations with a SEM of ±5%.

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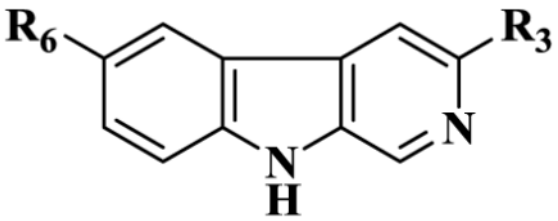
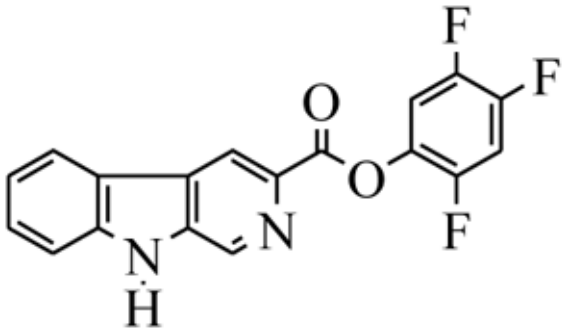
Table 2a

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



Ligands	R_6	R_3	α_1	α_2	α_3	α_4	α_5	α_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



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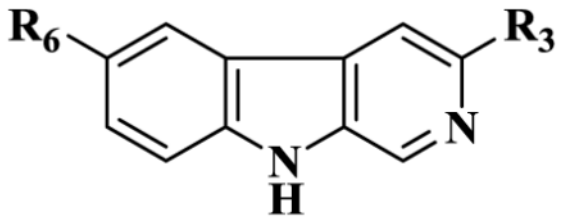
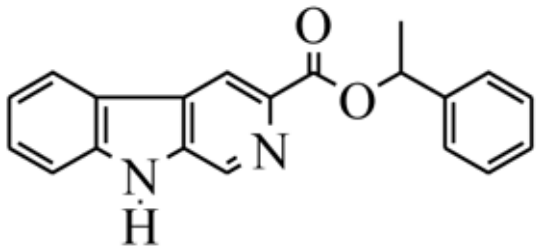


Ligands	R_6	R_3	α_1	α_2	α_3	α_4	α_5	α_6
26(CM-A-77)			33.5	1000	1000	1000	1000	3000

The affinity of compounds at $GABA_A$/BzR recombinant subtypes was measured by competition for [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%.

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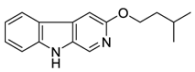
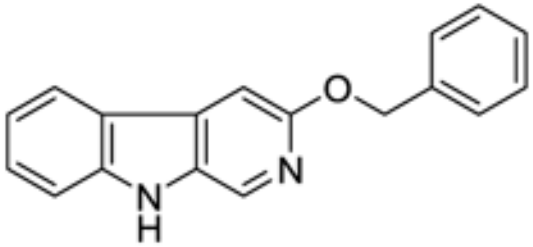
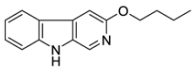
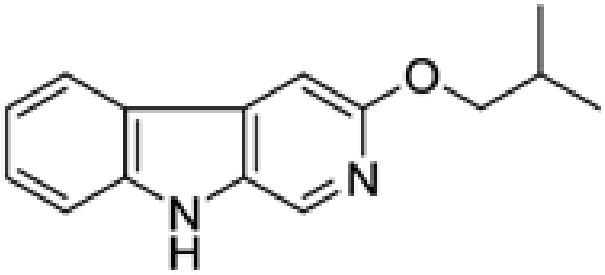
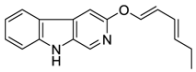
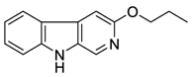
Table 2b

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

Ligands	α_1	α_2	α_3	α_5	α_6
 **34**	350.2	3000	3000	3000	10000
 **35**	830	3000	3000	10000	10000
 **36**	36.9	194	245	1000	1000
 **37**	24.9	123.6	139.2	1000	10000
 **38**	245	818	859	10000	10000
 **2**	5.3	52.3	68.8	591	1000

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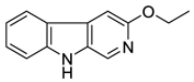
Ligands	α_1	α_2	α_3	α_5	α_6
 **39**	6.43	25.1	28.2	826	1000

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 $\alpha1\beta3\gamma2$, $\alpha2\beta3\gamma2$, $\alpha3\beta3\gamma2$, $\alpha4\beta3\gamma2$, $\alpha5\beta3\gamma2$ and $\alpha6\beta3\gamma2$.[119] Data represent the average of at least three determinations with a SEM of ±5%.

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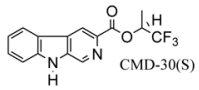
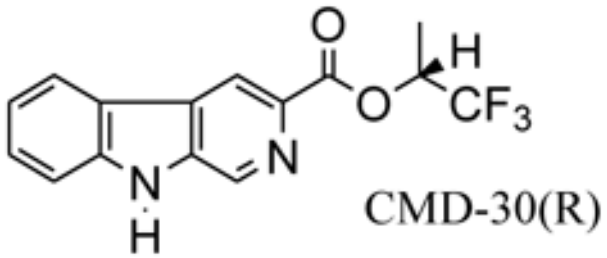
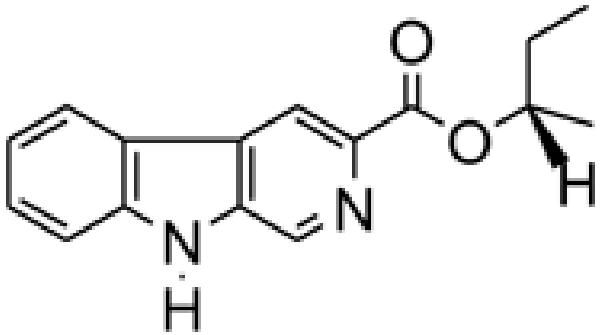
Table 3a

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	α_1	α_2	α_3	α_4	α_5	α_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

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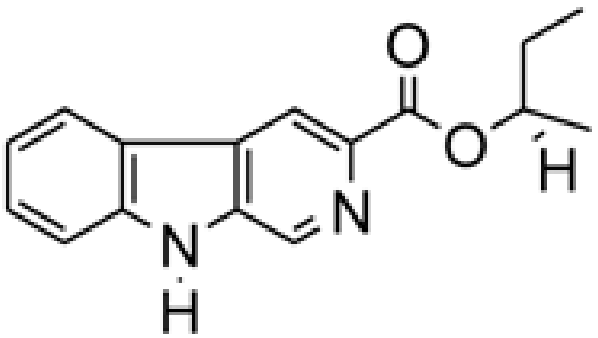
Ligands	α_1	α_2	α_3	α_4	α_5	α_6
24 	7.7	32.5	43	NA	69	>2000

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 $\alpha1\beta3\gamma2$, $\alpha2\beta3\gamma2$, $\alpha3\beta3\gamma2$, $\alpha4\beta3\gamma2$, $\alpha5\beta3\gamma2$ and $\alpha6\beta3\gamma2$.[119] Data represent the average of at least three determinations with a SEM of ±5%.

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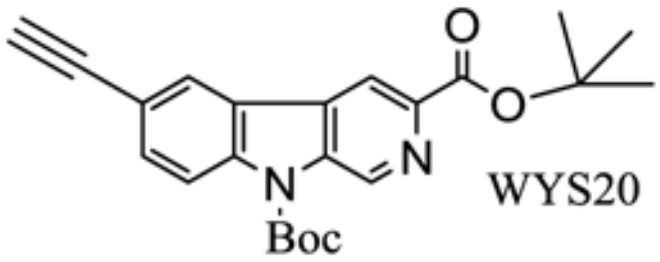
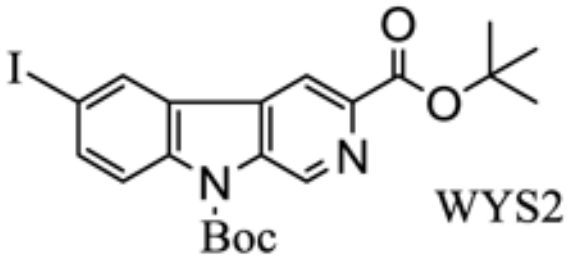
Table 3b

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		α_1	α_2	α_3	α_4	α_5	α_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%.