Synthesis and Pharmacological Properties of Novel 8-Substituted Imidazobenzodiazepines: High-Affinity, Selective Probes for α5-Containing $GABA_A$ Receptors[1]

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Received December 5, 1995[⊗]

The synthesis and pharmacological properties of imidazobenzodiazepines with both high affinity and selectivity for α5-containing $GABA_A$ receptors are described. Four of these compounds (**5**, **6**, **8**, and **9**) inhibited [^{3}H]flunitrazepam binding to recombinant $\alpha5\beta2\gamma2$ $GABA_A$ receptors with IC_{50} values between ∼0.4 and 5 nM. These compounds were ≥24−75-fold more selective for recombinant receptors containing α5 subunits compared to other, "diazepam-sensitive" $GABA_A$ receptors containing either α1, α2, or α3 subunits. Imidazobenzodiazepine **9** (used as the prototypical α5 selective ligand) inhibited [^{3}H]flunitrazepam binding to hippocampal membranes with high- and low-affinity components (IC_{50} 0.6 ± 0.2 and 85.6 ± 13.1 nM, respectively), representing ∼16% and ∼84% of the receptor pool. Inhibition of [^{3}H]flunitrazepam binding to cerebellar membranes with imidazobenzodiazepine **9** was best fitted to a single population of sites with an IC_{50} of 79.8 ± 18.3 nM. These imidazobenzodiazepines behaved as GABA negative ligands in recombinant $GABA_A$ receptors expressed in *Xenopus* oocytes and were convulsant in mice after parenteral administration. The relative potencies of flumazenil and zolpidem in blocking convulsions induced by **9** and DMCM, respectively, indicated that occupation of α5-containing $GABA_A$ receptors substantially contributed to the convulsant properties of acetylene analog **9**. These 8-substituted imidazobenzodiazepines (**5**, **6**, **8**, and **9**) should prove useful in examining the physiological roles of $GABA_A$ receptors bearing an α5 subunit and may also lead to the development of novel, subtype selective therapeutic agents.

Introduction

$GABA_A$ receptors mediate the actions of many pharmacologically useful agents, including benzodiazepines, barbiturates, and neuroactive steroids.[2,3] This heterogeneous family of ligand-gated ion channels is likely to assume a pentameric structure that may be assembled from 15 possible subunits (not including splice variants) which have been identified in the mammalian central nervous system.[4] On the basis of the degree of amino acid identity, these subunits have been grouped into five classes which each contain 1−6 variants (6α, 3β, 3γ, δ, and 2ρ).[5]

Subunit composition appears to be the primary determinant of both ligand affinity and efficacy at $GABA_A$ receptors.[6−8] The impact of subunit composition on drug action at both recombinant and wild type $GABA_A$ receptors is perhaps best characterized for the chemically diverse class of compounds referred to as benzodiazepine receptor (BzR) site ligands.[5,9] On the basis of studies in recombinant $GABA_A$ receptors, variation among α subunits appears to have the most profound impact on the affinities of diverse classes of BzR site ligands.[9,10] Substitution among γ subunits affects the affinity of a more circumscribed class of compounds,[11] but can dramatically alter ligand efficacy.[12] For example, prototypical 1,4-benzodiazepines such as diazepam and flunitrazepam possess nanomolar affinities for recombinant $GABA_A$ receptors containing α1, -2, -3, or -5 subunits, but are essentially inactive at recombinant receptors bearing α4 or α6 subunits.[13,14] Given the distinct neuroanatomical distribution of $GABA_A$ receptor subunits,[15] the potential exists for the development of subtype selective drugs with unique pharmacological profiles.

While there are some BzR site ligands which possess high affinities for recombinant $GABA_A$ receptors containing the α5 subunit,[16,17] these ligands are not very selective for α5-containing $GABA_A$ receptors relative to the other diazepam-sensitive isoforms. In contrast, the imidazobenzodiazepine Ro 15-4513 exhibited a 10−15-fold selectivity for recombinant $GABA_A$ receptors which contained an α5 as compared to α1, α2, or α3 subunits.[17] Both *in situ* hybridization and immunochemical studies indicate that the hippocampus is relatively enriched in α5 containing $GABA_A$ receptors compared to other brain areas.[15,18,19] While the physiological and pharmacological roles of specific $GABA_A$ receptor subtypes have not yet been evinced, the synthesis of subtype selective ligands may provide useful tools to clarify them. We now report the synthesis of a series of C(8)-substituted ligands that exhibit high affinity ($K_i \approx 0.4-5$ nM) and selectivity (up to ∼75.5-fold compared to other diazepam sensitive receptor isoforms) for α5-containing $GABA_A$ receptors. Moreover, the affinities of a prototypical α5 selective ligand *tert*-butyl 8-acetylenyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate for wild type $GABA_A$ receptors were consistent with the α5 selectivity observed in recombinant $GABA_A$ receptors. Several of these novel ligands were convulsants, consistent with their inverse agonist properties in recombinant α5-containing $GABA_A$ receptors.

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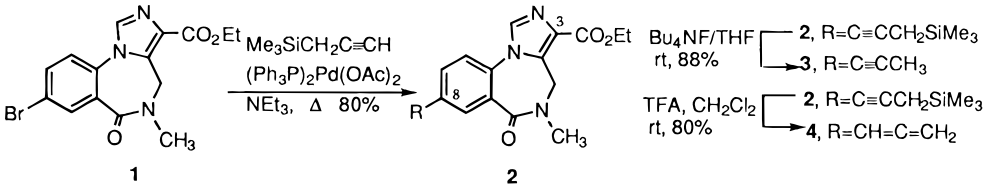
[⊗] Abstract published in *Advance ACS Abstracts,* April 1, 1996.

0022-2623/96/1839-1928$12.00/0

Scheme 1



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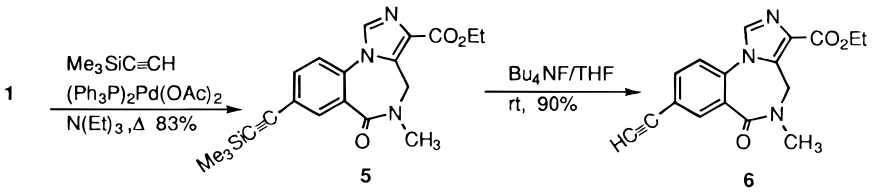
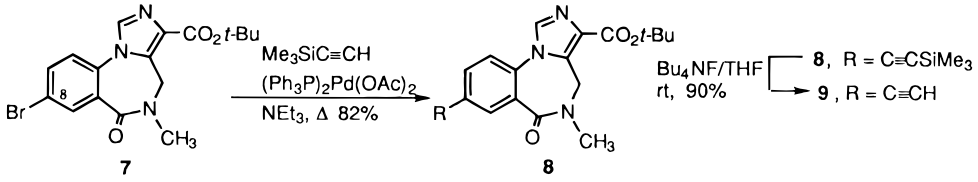
Scheme 2



5 6

Scheme 3



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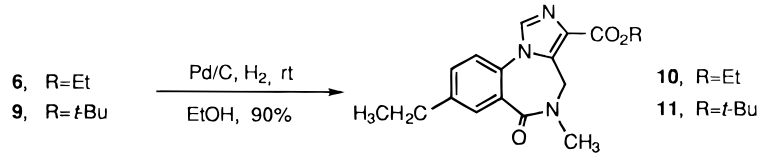
Scheme 4



On the basis of the potencies of zolpidem and Ro 15-1788 to block convulsions induced by DMCM or *tert*-butyl 8-acetylenylimidazobenzodiazepine (**9**), it is likely the convulsant properties of these novel imidazobenzodiazepines are mediated *via* occupation of α5-containing $GABA_A$ receptors.

Chemistry

The synthesis of 8-substituted imidazobenzodiazepines is outlined in Schemes 1−4. The starting 8-bromoimidazobenzodiazepine required for this study was prepared by the method of Gu et al.[20] A Heck type coupling reaction was employed to install the acetylene and allene functionality at position 8 of the imidazobenzodiazepine nucleus.[21] Thus, the benzodiazepine template **1** was coupled with propargyltrimethylsilane in the presence of bis(triphenylphosphine)palladium(II) acetate (10 mol %) to provide the C(8)-functionalized system **2** in 80% yield. Treatment of the trimethylsilyl analog **2** with Bu_4NF effected the desilylation to furnish the methylacetylene congener **3**. The conversion of **2** into allene **4** was effected in the presence of TFA presumably facilitated by β-silicon stabilization of a cationic transition state.[22]

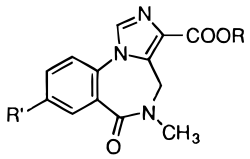
Employing the same strategy as described above, 8-bromoimidazobenzodiazepine **1** was coupled with (trimethylsilyl)acetylene to provide silylacetylene **5**, which in turn was treated with Bu_4NF to yield the acetylenylbenzodiazepine **6** (Scheme 2). Similarly, the related *tert*-butyl ester **9** was obtained by the desilylation of trimethysilyl-substituted benzodiazepine **8** which had been previously prepared from benzodiazepine **7** (Scheme 3). The 8-ethyl-substituted imidazobenzodiazepines **10** and **11** were obtained from the 8-acetylenic-substituted imidazobenzodiazepines **6** and **9**, respectively, in high yield *via* catalytic hydrogenation (Pd/C, H_2), as outlined in Scheme 4.

Results and Discussion

"Classical" 1,4-benzodiazepines exhibit remarkably similar clinical profiles, demonstrating anxiolytic, sedative, myorelaxant, anticonvulsant, amnestic, and respiratory depressant properties. This similarity may reflect a lack of selectivity among "diazepam-sensitive" $GABA_A$ receptors. Consistent with this hypothesis, 1,4-benzodiazepines such as diazepam and flunitrazepam exhibit similar affinities for recombinant $GABA_A$ receptors containing α1, α2, α3, or α5 subunits.[1,23] While the concept of designing subtype selective BzR ligands with unique pharmacological profiles has been recognized for over 15 years,[24] the potential for $GABA_A$ receptor heterogeneity could not have been either fully appreciated or exploited in those pioneering studies. The cloning, expression, and anatomical localization of multiple $GABA_A$ subunits has facilitated both the identification and design of subtype selective compounds in order to develop agents that possess either a narrower range of clinical activities or fewer undesirable effects than currently used drugs acting at Bz modulatory sites.

The rationale for designing α5 selective ligands was based in part on the restricted neuroanatomical distribution of this subunit compared to the other α subunits (α1, α2, α3) that constitute "diazepam-sensitive" $GABA_A$ receptors. Thus, on the basis of both *in situ* hybridiza-

Table 1. Affinities of Novel Imidazobenzodiazepines at Recombinant $\alpha x\beta 2\gamma 2$ $GABA_A$ Receptors[a]



			K_i (nM)					
compd	R	R′	α1	α2	α3	α5	α6	α1/α5
2	CH_2CH_3	$C{\equiv}CCH_2Si(CH_3)_3$	>300	>300	>300	>300	>300	
3	CH_2CH_3	$C{\equiv}CCH_3$	10.1	22.2	16.5	1.7	>100	6.0
4	CH_2CH_3	$CH{=}C{=}CH_2$	3.8	7.2	4.1	1.1	44.3	3.4
5	CH_2CH_3	$C{\equiv}CSi(CH_3)_3$	121.1	141.9	198.4	5.0	113.7	24
6	CH_2CH_3	$C{\equiv}CH$	28.4	21.4	25.8	0.5	28.8	60
7	$C(CH_3)_3$	Br	11.4	10.7	9.2	0.5	9.4	24
8	$C(CH_3)_3$	$C{\equiv}CSi(CH_3)_3$	197	142.6	255	2.61	58.6	75.5
9	$C(CH_3)_3$	$C{\equiv}CH$	26.9	26.3	18.7	0.4	5.1	67.3
10	CH_2CH_3	CH_2CH_3	20.4	27	26.1	1.5	176	13.8
11	$C(CH_3)_3$	CH_2CH_3	14.8	56	25.3	1.7	22.9	8.6
Ro 15-4513	CH_2CH_3	N_3	3.3	2.6	2.5	0.26	3.8	12.7

[a] K_i values represent the mean of two determinations which differed by less than 10%. Data were generated using Ltk^- cell membranes expressing human $\alpha x\beta 2\gamma 2$ receptors. [^{3}H]Ro 15-1788 and [^{3}H]Ro 15-4513 (for cells expressing $\alpha 6\beta 2\gamma 2$ receptors) were used as radioligands at a final concentration of 1−2 nM.[1]

tion and immunoprecipitation studies, $GABA_A$ receptors containing the α5 subunit are present in hippocampus and to a lesser extent in cerebral cortex, but are found in very low abundance in most other brain regions.[15,18,19] Moreover, a lead compound, the imidazobenzodiazepine Ro 15-4513, had been shown to exhibit a 10−15-fold higher affinity for recombinant α5-containing receptors as compared to other "diazepam-sensitive" receptor isoforms.[23,25,31]

As illustrated in Table 1, prototypical imidazobenzodiazepines such as Ro 15-4513 and Ro 15-1310[1] exhibited high affinities for all five recombinant $GABA_A$ receptor subtypes, together with a moderate selectivity for the $\alpha 5\beta 2\gamma 2$ subtype (∼8−10-fold). When position 8 of Ro 15-4513 was substituted with a fluorine atom, the resulting ligand [flumazenil (Ro 15-1788)] lost selectivity for the $\alpha 5\beta 2\gamma 2$ subtype.[1] This indicated that ligands which have substituents of varied size which fit into the lipophilic region L_2 of the inclusive pharmacophore[26] might exhibit selectivity at α5-containing receptors as compared to other receptor subtypes.

This hypothesis was supported by examination of the affinities of the imidazobenzodiazepines depicted in Table 1 in recombinant $GABA_A$ receptors.[31] For example, when the imidazobenzodiazepine nucleus was substituted at position 8 with an acetylenic group (see **6**), the potency at $\alpha 5\beta 2\gamma 2$ receptor subtypes was about 50 times higher than at the other four $GABA_A$ receptor subtypes examined (see Table 1). When this same position was substituted with a (trimethylsilyl)acetylene moiety (**5** and **8**), the ligands also exhibited excellent selectivity for the $\alpha 5\beta 2\gamma 2$ subtype. While several of the other derivatives (**3**, **4**, **10**, and **11**) retained high affinities for $GABA_A$ receptors, reduction of the ethynyl function, or increasing the alkyl function at C(8) by one carbon atom (regardless of the degree of unsaturation), reduced selectivity for α5 containing receptors (see **3**). From comparison of the *in vitro* affinities of α5 selective ligands **5**, **6**, **8**, and **9** (Table 1) to that of flumazenil (Ro 15-1788) or Ro 15-1310,[1] it is evident that the larger substituents at C(8) confer selectively to these former ligands for recombinant $\alpha 5\beta 2\gamma 2$ receptors. To the best of our knowledge, these novel C(8)-substituted imidazobenzodiazepines represent the most selective ligands at the $\alpha 5\beta 2\gamma 2$ subtype reported to date.

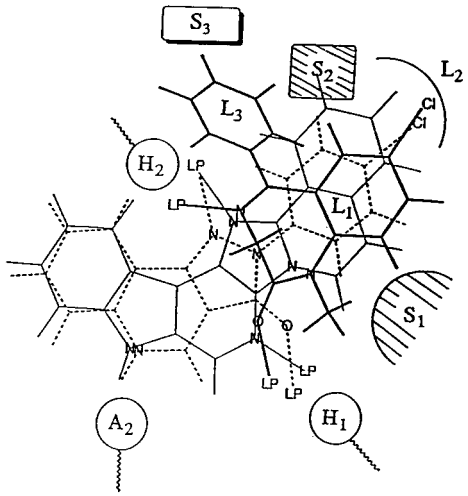


Figure 1. The pyrazolo[3,4-*c*]quinolin-3-one ligand CGS-9896 (dotted line), diazepam (thick line), and diindole (thin line) fitted to a schematic representation of the inclusive pharmacophore model for the BzR. The sites H_1 and H_2 designate hydrogen bond donator sites on the receptor protein while A_2 represents a hydrogen bond acceptor site. Receptor descriptors S_1, S_2, and S_3 are regions of negative steric repulsion.

Examination of the SAR data illustrated in Table 1 suggests that the lipophilic pocket designated L_2 in the inclusive pharmacophore (see Figure 1)[26,28] is smaller in $\alpha 1\beta 2\gamma 2$ receptors than that present in the related $\alpha 5\beta 2\gamma 2$ subsite. Support for this statement derives from comparison of the affinities of **5** [α1 (121 nM), α5 (5 nM)] or **8** [α1 (197 nM), α5 (2.6 nM)] to Ro 15-1788 [α1 (0.8 nM), α5 (0.6 nM)].[1] Consequently, it is likely the attractive lipophilic−lipophilic interactions between the methyl group on zolpidem[1] and the smaller lipophilic pocket L_2 in the $\alpha 1\beta 2\gamma 2$ site are responsible, at least in part for the high affinity of zolpidem at the Bz_1 ($\alpha 1\beta 2\gamma 2$) site. However, L_2 appears to be larger in recombinant $\alpha 5\beta 2\gamma 2$ receptors, and this attractive interaction is absent, resulting in a very low affinity of zolpidem at recombinant $\alpha 5\beta 2\gamma 2$ receptors.[1,16] In the present study, a C(8) substituent such as the (trimethylsilyl)acetylene

Table 2. Convulsant and Proconvulsant Activity of α5 Selective Imidazobenzodiazepines[a]

compd	maximum % convulsing	CD_{50} (mg/kg)
5	85	70.9
6	70	24
8	22	2.3[b]
9	80	5.9

[a] Animals were injected with graded doses as described in the Experimental Section. The maximum percent convulsing is reported for each compound. The highest doses used were 40 mg/kg, except compound **5** which was tested at doses up to 80 mg/kg. Convulsant potency was assessed by the percentage of animals seizing within 10 minutes after receiving a single ip injection of the compound. CD_{50} was defined as the dose at which half of the maximal number of animals seized. [b] Since only a maximum of 22% of the animals seized with the maximum dose of **8**, the proconvulsant potency (PD_{50}) was also determined for this compound. PD_{50} was defined as the dose at which 50% of the animals convulsed after a single ip injection of the compound 15 min after receiving PTZ [40 mg/kg (ip) $N = 5-10$ in each group]. This dose of PTZ did not produce convulsions in saline pretreated mice. CD_{50} and PD_{50} were determined by nonlinear regression curve-fitting using GraphPad Inplot.

moiety (**5** and **8**) is large and may fit better in the lipophilic pocket L_2 of α5 bearing receptors, resulting in high-affinity binding. Presumably, this substituent is too large to readily fit in L_2 of the corresponding α1β2γ2 sites.

Several of these compounds (**8** and **9**) were GABA-negative ligands in recombinant receptors expressed in *Xenopus* oocytes[1] and, as predicted from this profile,[29] were convulsants in mice (Table 2). Using imidazobenzodiazepine **9** as a prototype for additional *in vitro* and *in vivo* studies, a profile consistent with the α5 selectivity demonstrated in recombinant receptors was obtained in wild type receptors. Thus, competition studies with **9** in hippocampal membranes indicated the presence of both high- (IC_{50} 0.6 ± 0.2 nM) and low-affinity (IC_{50} 85.6 ± 13.1 nM) sites, while parallel studies revealed one site (IC_{50} 79.8 ± 18.3 nM) in cerebellar membranes (Figure 2). Moreover, the proportion of high-affinity sites for **9** in hippocampal membranes (16 ± 4% of the receptor pool) is in excellent agreement with the percentage of α5-containing $GABA_A$ receptors in rat and bovine hippocampus estimated using subunit specific antibodies.[18,19,25] The agreement between the IC_{50} values of **9** to the low-affinity hippocampal Bz binding sites and those in the cerebellum is also consistent with the lack of selectivity of this compound among α1-, α2-, and α3-containing recombinant receptors (Table 1).

Since imidazobenzodiazepine **9** appeared to exhibit selectivity for both recombinant and wild type $GABA_A$ receptors containing an α5 subunit, a series of experiments were designed to determine the relative contribution of α5-containing receptors to the convulsant actions of this compound (Figure 3). The β-carboline DMCM was selected for comparison with imidazobenzodiazepine **9** since it possesses high affinity but little selectivity among diazepam-sensitive $GABA_A$ receptors.[1] Flumazenil, a GABA-neutral ligand which binds with equal affinity among recombinant diazepam-sensitive $GABA_A$ receptors,[1] was nearly equipotent in blocking the convulsant actions of **9** and DMCM (ED_{50} 3.8 and 2.3 mg/kg, respectively). In contrast, zolpidem was ~18-fold less potent in blocking **12** than DMCM-induced convulsions (ED_{50} 7.0 and 0.4 mg/kg, respectively). This observation is consistent with the hypothesis that the convulsant action of **9** is in large part mediated through

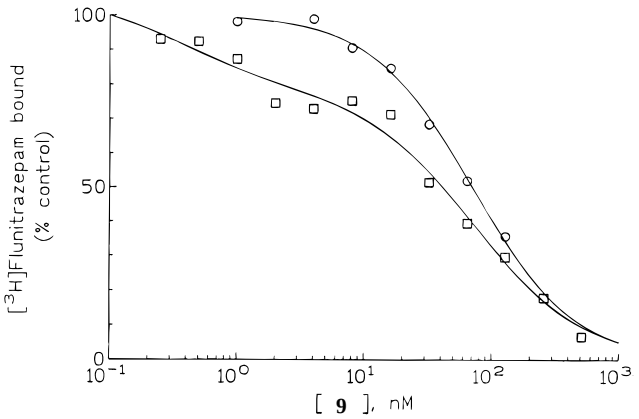


Figure 2. Inhibition of [^{3}H]flunitrazepam binding by imidazobenzodiazepine **9** in cerebellar (circles) and hippocampal (squares) membranes. The inhibition of [^{3}H]flunitrazepam binding by **9** in hippocampal membranes was best fit to a two site competition curve (*F* test). In this representative experiment, the IC_{50} values for **9** in hippocampal membranes were 0.33 nM (high affinity) and 70.7 nM (low affinity), while the IC_{50} value in cerebellar membranes was 67.2 nM (Hill slope = −1.03). Each point represents the average of a replicate determination. In results from four experiments, hippocampal high affinity (IC_{50} 0.6 ± 0.2 nM, mean ± SEM) and low affinity (IC_{50} 85.6 ± 13.1 nM) components represented 16 ± 4% and 84 ± 4% of the receptor pool, respectively. The IC_{50} value of **9** in cerebellar membranes was 79.8 ± 18.3 nM, Hill slope = −0.96 ± 0.07. Parameter estimates were calculated using GraphPad Inplot.

occupation of α5-containing $GABA_A$ receptors, since the affinity of zolpidem at recombinant α5 receptors is >10 μM.[16] On the basis of this very low affinity of zolpidem, it might be argued that if **9** were producing convulsions through α5-containing $GABA_A$ receptors, then the anticonvulsant potency of zolpidem should be even lower than was observed in the present study (Figure 3). However, zolpidem binds with relatively high affinity to both recombinant and wild type α_1-containing GABA receptors[16] and with moderate affinities at α2 and α3 $GABA_A$ receptor isoforms.[1] Since α1β2−3γ2-containing receptors appear to be the predominant form of GABA receptors in brain,[29] it is possible that zolpidem acts as an anticonvulsant by blocking the propagation of seizures through increasing the GABAergic tone at these receptors. Consistent with this hypothesis, the ED_{50} of zolpidem in blocking pentylenetetrazole or MES-induced convulsions was ~9 mg/kg.[30]

While imidazobenzodiazepine **9** had the highest affinity for α5-containing recombinant receptors and was the most potent convulsant among the newly synthesized derivatives examined (Table 1), the convulsant potencies of the four derivatives tested did not strictly correlate with their affinities at recombinant α5-containing receptors (Table 2). This apparent discrepancy may be related to pharmacokinetic factors, since it was more difficult to maintain the trimethylsilyl derivatives (**8** and **11**) in uniform suspension. Measurement of brain levels together with *in vivo* binding studies could aid in resolving this issue. It is also noteworthy that none of these derivatives were convulsant in 100% of the animals (even at doses as high as 40−80 mg/kg), while DMCM routinely produced convulsions in 100% of the mice tested at 7.5 mg/kg (Figure 3). Among a series of structurally related β-carbolines, it is the ability to act as GABA-negative ligands in wild

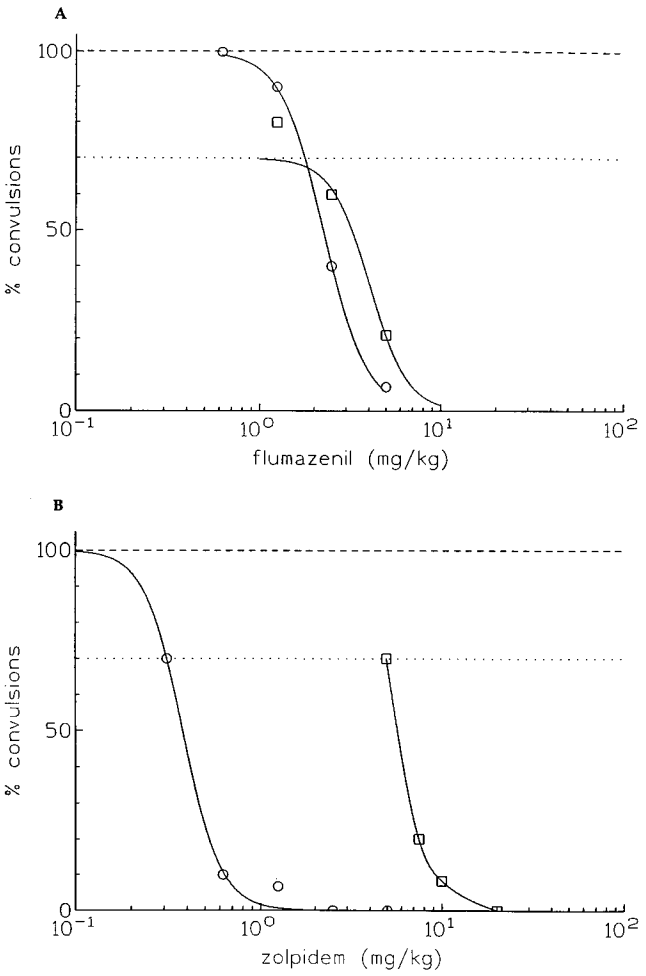


Figure 3. Anticonvulsant activity of flumazenil and zolpidem against convulsions induced by DMCM (circles) or imidazobenzodiazepine **9** (squares). Mice were administered vehicle, flumazenil, or zolpidem and challenged 10 min later with DMCM (7.5 mg/kg, circles) or **9** (20 mg/kg, squares). Dashed lines represent the maximum percentage of mice convulsing at this dose of DMCM, dotted lines represent the maximum percentage of mice convulsing at this dose of imidazobenzodiazepine **9** ($N = 5-10$ mice at each concentration). The ED_{50} values of flumazenil versus DMCM and **9** were 2.3 and 3.8 mg/kg, respectively. The ED_{50} values of zolpidem versus DMCM and **9** were 0.4 and 7.0 mg/kg, respectively. Curves were fitted by nonlinear regression.

type receptors which appears to determine convulsant efficacy.[29] At recombinant α5-containing receptors expressed in *Xenopus* oocytes, **9** is as efficacious as DMCM as a GABA-negative ligand,[1] while **8** (the least potent convulsant) is approximately half as efficacious. On the basis of these findings, it is likely that the failure of even high doses of these α5 selective ligands to produce convulsions in 100% of the mice may be related to differential efficacies of these compounds among wild type $GABA_A$ receptors. Preliminary results with **5** and **6** at recombinant α1-containing $GABA_A$ receptors are consistent with this hypothesis.

Conclusion

Examination of the studies described above illustrates that modification of imidazobenzodiazepines at position 8 can profoundly affect the affinity and selectivity at both wild type and recombinant $GABA_A$ receptor subtypes. Substitution of an acetylenic function at position 8 of the imidazobenzodiazepine nucleus has provided the most selective ligand (**9**) reported to date for the α5-containing $GABA_A$ receptor. The action *in vivo* of these ligands resembled that of an inverse agonist, such as DMCM. Further research directed toward the synthesis and development of ligands with improved selectivity for α5-containing GABA receptors, but with differing efficacies may provide additional insights into the physiological functions subserved by this GABA receptor subtype. Moreover, these compounds serve as templates for the description of a pharmacophore for this receptor subtype[20,26,31] that may ultimately result in compounds with a more restricted and beneficial pharmacological profile than agents presently available. For example, the amnestic properties of classical 1,4-benzodiazepines are well described and are sometimes therapeutically useful as an adjunct to general anesthesia. Nonetheless, the amnestic properties of these compounds are often considered undesirable. Give the high density of α5-containing $GABA_A$ receptors in hippocampus (a structure critical to learning and memory processes), the development of GABA-neutral, selective α5 ligands may be of interest to both basic and clinical research.

Experimental Section

In Vitro. Radioligand binding to brain membranes was performed using modifications of previously described procedures.[27,31-34] Male Sprague−Dawley rats (200−250 g) were killed by decapitation. Hippocampi and cerebella were removed and disrupted in 50 volumes of Tris-citrate buffer (50 mM, pH 7.4) using a Brinkman Polytron (20 s, setting 7). Tissues were centrifuged for 20 min (4 °C) at 20000*g*. The supernatant was discarded and the tissue pellet resuspended in an equal volume of buffer. This washing procedure was repeated five times. Tissue homogenates were stored at −70 °C until used. Incubations (1.0 mL) consisted of tissue suspension (0.1 mL, 50−70 μg of protein), 0.1 mL of NaCl solution (2.0 M), 0.05 mL of [^{3}H]flunitrazepam (final concentration, ~1 nM, specific activity 82 Ci/mmol), and drugs and/or buffer to equal volume of Ro 15-1788 (final concentration 10 μM). Incubations (0−4 °C) were initiated by addition of radioligand and terminated after 120 min by rapid filtration under vacuum through GF/B filters with two 5 mL washes of ice-cold buffer. Data were evaluated using InPLot 4.0 (Graphpad, San Diego, CA). IC_{50} values were determined with 8−12 concentrations of test compound.

In Vivo. Adult male NIH/Swiss mice (~30 g) were injected intraperitoneally (ip) with graded doses of test compounds (0.1 mL) or an equal volume of vehicle. Drugs were suspended in 10% diluted Emulphor:90% saline, followed by gentle warming in a water bath and a brief sonication. Suspensions were then vortexed immediately prior to injection. The derivatives containing an ethynyltrimethylsilyl substituent at C-8 (**5** and **8**) were more difficult to maintain in uniform suspension than the derivatives containing ethynyl substituents at C-8 (**6** and **9**). **Convulsant Activity.** Groups of mice were injected with the test compound in graded doses or an equal volume of vehicle. Subjects were placed in individual plastic cages and observed for 10 min postinjection. Tonic and clonic convulsions with loss of righting reflex were considered convulsant activity; mild myoclonic jerks and Straub tail were not scored as convulsions. **Proconvulsant Activity.** Fifteen minutes after injection of **8** or an equal volume of vehicle, mice were injected with PTZ (40 mg/kg) to assess proconvulsant activity. This dose of PTZ routinely produced convulsions in <10% of vehicle treated mice. Animals were observed for 10 min as described above. **Blockade of DMCM- and 9-Induced Convulsions.** Groups of mice were injected with graded doses of zolpidem, flumazenil, or an equal volume of vehicle. Ten minutes later, the mice were injected with maximally effective convulsant doses of either DMCM (7.5 mg/kg) or **9** (20 mg/kg). Animals were then observed for the presence of convulsions as described.

Materials. [^{3}H]Flunitrazepam (specific activity 82 Ci/mmol) was obtained from New England Nuclear, Boston, MA; Emulphor from Rhone-Poulenc, Cranbury, NJ; zolpidem from Synthelabo Recherche; flumazenil (Ro 15-1788) from Hoffman-La Roche, Nutley, NJ; PTZ (pentylenetetrazole) from ICN-K&K Laboratories, Plainview, NY; DMCM (methyl 6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate) from Research Biochemicals Incorporated, Natick, MA; Sprague−Dawley rats from Taconic Farms; and NIH-Swiss mice from the Frederick South Facility of the National Cancer Institute, Frederick, MD. All animals were housed in AALAC-approved facilities at the NIH with 12-h light−dark cycles and free access to food and water.

Melting points were taken on a Thomas-Hoover melting point apparatus or an Electrothermal model IA8100 digital melting point apparatus and are reported uncorrected. The ^{1}H NMR spectra were recorded on a Bruker 250-MHz multiple-probe instrument or a GE 500-MHz spectrometer. Infrared spectra were recorded on a Nicolet Dx FTIR DX V5.07 spectrometer or a Mattson Polaris IR-10400 instrument. Low-resolution mass spectral data (EI/CI) were obtained on a Hewlett-Packard 5985 B GC−mass spectrometer. Microanalyses were performed on a Perkin-Elmer 240C carbon, hydrogen, and nitrogen 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 indicated. The starting 1,4-benzodiazepine **1** was prepared by the method of Gu et al.[20]

Ethyl 8-[3-(Trimethylsilyl)propynyl]-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (2). A mixture of benzodiazepine **1**[20] (0.12 g, 0.33 mmol), propargyltrimethylsilane (0.56 mmol, 0.084 mL), and bis(triphenylphosphine)palladium(II) acetate (0.025 g, 0.033 mmol) in 30 mL of anhydrous triethylamine was heated to reflux under argon. After 10 h, the mixture was cooled to room temperature, and the precipitate which resulted was removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was treated with 30 mL of saturated aqueous $NaHCO_3$ (30 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined extracts were washed with brine and dried (K_2CO_3). After removal of solvent under reduced pressure, the residue was purified by flash chromatography (silica gel, EtOAc) to afford **2** as yellow crystals (0.10 g, 80%): mp 121−122 °C; IR (KBr) 3114, 2959, 2214, 1722, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.15 (s, 9H), 1.42 (t, 3H, J = 7.1 Hz), 1.71 (s, 2H), 3.19 (s, 3H), 4.37−4.45 (m, 3H), 5.14 (br, 1H), 7.29 (d, 1H, J = 8.3 Hz), 7.55 (d, 1H, J = 8.3 Hz), 7.84 (s, 1H), 8.00 (s, 1H); MS (EI) *m/e* 395 (M$^+$, 100), 321 (10), 277 (45), 248 (15), 236 (17), 221 (11), 153 (10), 139 (14), 114 (14), 113 (12). Anal. ($C_{21}H_{25}N_3O_3Si$) C, H, N.

Ethyl 8-propynyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (3). A solution of **2** (0.05 g, 0.13 mmol) in THF (15 mL) was treated with Bu_4NF (1.0 M in THF, 0.16 mL). The mixture which resulted was stirred for 1 h at room temperature, after which the mixture was added to H_2O (10 mL) and extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine (15 mL) and dried (K_2CO_3). After removal of solvent under reduced pressure, the residue was purified by a wash column (silica gel, EtOAc) to give benzodiazepine **3** as a light yellow solid (0.037 g, 88%): mp 138−139 °C; IR (KBr) 3050, 2900, 2200, 1750, 1675 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, J = 7.1 Hz), 2.06 (s, 3H), 3.22 (s, 3H), 4.05−4.14 (m, 3H), 5.22 (br, 1H), 7.32 (d, 1H, J = 8.3 Hz), 7.58 (d, 1H, J = 8.4 Hz), 7.85 (s, 1H), 8.04 (s, 1H); MS (EI) *m/e* 323 (M$^+$, 30), 278 (22), 277 (33), 250 (39), 249 (100), 221 (30), 168 (22), 115 (34), 113 (34), 101 (19). Anal. ($C_{18}H_{17}N_3O_3$) C, H, N.

Ethyl 8-Allenyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (4). Benzodiazepine **2** (0.05 g, 0.13 mmol) in CH_2Cl_2 (2 mL) was treated with excess TFA which was added to the solution at room temperature. The mixture was then stirred for 15 h, after which aqueous NH_4OH was added to neutralize the acid. The organic layer was separated and dried over K_2CO_3. After the solvent was removed under reduced pressure, the residue was purified by a wash column (silical gel, EtOAc) to afford **4** as a light yellow solid (0.033 g, 80%): mp 196−197 °C; IR (KBr) 3100, 3000, 1950, 1718, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, J = 7.1 Hz), 3.20 (s, 3H), 4.35−4.43 (m, 3H), 5.13 (br, 1H), 5.21 (d, 2H, J = 6.7 Hz), 6.19 (t, 1H, J = 6.7 Hz), 7.32 (d, 1H, J = 8.3 Hz), 7.50 (d, 1H, J = 8.3 Hz), 7.84 (s, 1H), 7.90 (s, 1H); MS (EI) *m/e* 323 (M$^+$, 33), 312 (29), 311 (21), 283 (55), 277 (96), 249 (53), 239 (100), 183 (30), 114 (37), 103 (43). Anal. ($C_{18}H_{17}N_3O_3$) C, H, N.

Ethyl 8-[(trimethylsilyl)ethynyl]-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (5) was prepared in 83% yield from **1** and (trimethylsilyl)-acetylene using the same procedure as described for compound **2**. The ligand **5** was obtained as a yellow solid: mp 107−108 °C; IR (KBr) 3109, 2966, 2160, 1734, 1650 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.24 (s, 9H), 1.43 (t, 3H, J = 7.1 Hz), 3.22 (s, 3H), 4.42 (m, 3H), 5.17 (br, 1H), 7.35 (d, 1H, J = 8.3 Hz), 7.66 (d, 1H, J = 8.3 Hz), 7.88 (s, 1H), 8.14 (s, 1H); MS (EI) *m/e* 381 (M$^+$, 46), 335 (40), 308 (32), 307 (100), 278 (13), 277 (23), 183 (12), 160 (14), 146 (44), 132 (20). Anal. ($C_{20}H_{23}O_3N_3Si$) C, H, N.

Ethyl 8-ethynyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (6) was prepared in 90% yield from **5** using the same procedure as described for the preparation of **3**. The product **6** was obtained as light yellow crystals: mp 206−207 °C; IR (KBr) 3239, 3115, 2979, 2104, 1701 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, J = 7.1 Hz), 3.21 (s, 1H), 3.23 (s, 3H), 4.37−4.43 (m, 3H), 5.27 (br, 1H), 7.37 (d, 1H, J = 8.3 Hz), 7.69 (d, 1H, J = 8.3 Hz), 7.87 (s, 1H), 8.16 (s, 1H); MS (EI) *m/e* 309 (M$^+$, 39), 264 (17), 263 (60), 236 (34), 235 (100), 221 (15), 207 (25), 195 (12), 180 (9), 154 (12). Anal. ($C_{17}H_{15}O_3N_3$) C, H, N.

***tert*-Butyl 8-[(trimethylsilyl)ethynyl]-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (8)** was prepared in 82% yield from **7** and (trimethylsilyl)acetylene using the same procedure as described for **5**. The product was obtained as a dark yellow solid: mp 199−200 °C; IR (KBr) 2971, 2156, 1723, 1662, 1500 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.25 (s, 9H), 1.63 (s, 9H), 3.22 (s, 3H), 4.31 (br, 1H), 5.11 (br, 1H), 7.32 (d, 1H, J = 8.2 Hz), 7.64 (d, 1H, J = 8.2 Hz), 7.84 (s, 1H), 8.13 (s, 1H); MS (EI) *m/e* 409 (M$^+$, 4), 354 (19), 353 (82), 335 (45), 307 (100), 279 (23), 251 (16), 143 (13), 160 (31), 107 (14). Anal. ($C_{22}H_{27}N_3O_3Si$) C, H, N.

tert-Butyl 8-ethynyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (9) was prepared in 90% yield from **8** and Bu_4NF using the same procedure as described for **3**. The product was obtained as a yellow solid: mp 213−214 °C; IR (KBr) 3223, 2976, 2106, 1722, 1644 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.63 (s, 9H), 3.20 (s, 1H), 3.23 (s, 3H), 4.32 (br, 1H), 5.15 (br, 1H), 7.35 (d, 1H, J = 8.3 Hz), 7.69 (d, 1H, J = 8.3 Hz), 7.85 (s, 1H), 8.16 (s, 1H); MS (EI) *m/e* 337 (M$^+$, 2), 282 (15), 281 (78), 264 (31), 263 (56), 235 (100), 229 (22), 207 (36), 138 (16), 101 (19). Anal. ($C_{19}H_{19}N_3O_3$) C, H, N.

Ethyl 8-Ethyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (10). Acetylene **6** (0.118 g, 0.39 mmol) was dissolved in EtOH (30 mL) after which Pd/C (0.018 g) was added in solution at room temperature. The slurry was stirred for 4 h under an atmosphere of H_2. The catalyst was filtered off, and the EtOH was removed under reduced pressure to furnish a residue. This material was purified by flash chromatography (silical gel, EtOAc) to provide **10** (0.11 g, 90%) as white crystals: mp 124−125 °C; IR (KBr) 3100, 2975, 1710, 1600, 1500 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 3H, J = 7.6 Hz), 1.43 (t, 3H, J = 7.1 Hz), 2.75 (q, 2H, J = 7.6, 15 Hz), 3.24 (s, 3H), 4.38−4.44 (m, 3H), 5.14 (br, 1H), 7.32 (d, J = 8.2 Hz), 7.44 (d, J = 8.2 Hz), 7.82 (s, 1H), 7.89 (s, 1H); MS (EI) *m/e* 313 (M$^+$, 30), 277 (28), 267 (37), 240 (30), 239 (100), 211 (17), 199 (20), 183 (14), 155 (7), 142 (7). Anal. ($C_{17}H_{19}N_3O_3$) C, H, N.

***tert*-Butyl 8-ethyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (11)** was prepared in 90% yield from acetylene **9** using the same procedure described for benzodiazepine **10**. The *tert*-butyl

congener **11** was obtained as white crystals: mp 156−157 °C; IR (KBr) 3100, 2980, 1740, 1600, 1500 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (t, 3H, $J = 7.5$ Hz), 1.62 (s, 9H), 2.74 (q, 2H, $J = 7.6$, 15 Hz), 3.22 (s, 3H), 4.35 (br, 1H), 5.09 (br, 1H), 7.30 (d, 1H, $J =$ 8.2 Hz), 7.43 (d, 1H, $J = 8.2$ Hz), 7.85 (s, 2H); MS (EI) *m/e* 341 (M^+, 2), 285 (68), 268 (24), 2 67 (47), 239 (100), 211 (31), 184 (10), 142 (11), 115 (11), 103 (16). Anal. ($C_{19}H_{23}N_3O_3$) C, H, N.

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JM950887N