Synthesis of Novel Imidazobenzodiazepines as Probes of the Pharmacophore for "Diazepam-Insensitive" $GABA_A$ Receptors[1]

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The syntheses of a series of novel imidazobenzodiazepines and their affinities for diazepam sensitive (DS) and diazepam insensitive (DI) $GABA_A$ receptors are described. Imidazobenzodiazepines belong to one of the very few chemical families which exhibit high to moderate potency for DI $GABA_A$ receptors. Although imidazobenzodiazepines such as Ro 15-4513, **20**, are the most potent DI $GABA_A$ receptor ligands described to date, their selectivity for DI versus DS $GABA_A$ receptors is only marginal. Previous structure–activity relationship (SAR) studies of imidazobenzodiazepines have indicated that the 3- and 8-positions are critical for high-affinity binding to DI $GABA_A$ receptors (*J. Med. Chem.* **1993**, *36*, 479–490. *J. Med. Chem.* **1993**, *36*, 1001–1006. *J. Med. Chem.* **1993**, *36*, 1820–1830). In order to determine why the ester function is critical to high affinity at the DI site, we have synthesized several derivatives which have substituents other than an ester at the C(3) position including 3-alkyl-, 3-alkylketo-, 3-alkyl ether, and 3-dialkylamino-substituted imidazobenzodiazepines. The SAR analysis of these compounds when combined with that of several pyrazoloquinolinones indicates that interactions at H1 and L1 as well as interactions at H2 *anti* to the imidazole N(2) and at a lipophilic pocket (labeled LDi) about the 3-position are required in order for imidazobenzodiazepines to exhibit selectivity and high affinity for DI $GABA_A$ receptors. Furthermore, the imidazobenzodiazepines substituted with an electron-donating group (alkoxy function) at position 8 revealed that the change of the substituent at C(8) from an electron-withdrawing to a donating function did not substantially alter either ligand affinity or selectivity for DI $GABA_A$ receptors. Thus, a pharmacophore is proposed for DI $GABA_A$ receptor ligands, which is characterized by the requirement of a lipophilic pocket LDi about the C(3) position of imidazobenzodiazepines. Using this model, two pyrazoloquinolinone derivatives were designed and synthesized. Their affinities and selectivities for DI $GABA_A$ receptors are consistent with those predicted by the DI $GABA_A$ receptor pharmacophore. In addition, examination of the *in vitro* binding data of 3-alkyl ether analogs confirms that the *anti* conformation of the ester group at the C(3) position of imidazobenzodiazepines (Ro15-4513, **20** series) is preferred at both DI and DS $GABA_A$ receptors. This constitutes the first evidence (other than molecular modeling) to support the auxillary involvement of H2 at the DI site and is important with regard to the synthesis of other DI $GABA_A$ receptor selective ligands in the future. Comparison of the included volume developed here for the DI site vs the included volume for the DS site clearly demonstrates that the DI site is a smaller (subsite) binding cleft than the DS site and is clearly devoid of most of lipophilic area L3 (Figure 6b).

Introduction

$GABA_A$ receptors are a heterogeneous family of ligand-gated chloride ion channels that constitute the major inhibitory neurotransmitter system in the mammalian central nervous system (CNS).[2] The allosteric modulation of GABA binding by 1,4-benzodiazepines and related compounds elicits a wide range of pharmacological effects including sedation, muscle relaxation, anxiolytic, and anticonvulsant activities.[2] Such drug responsive $GABA_A$ receptors are constituted as hetero-oligomers containing multiple α, β, γ, δ, and ϱ subunits.[3–5] The DI $GABA_A$ receptors represent ~25–30% of cerebellar $GABA_A$ receptors but are a very minor constituent (0–5%) in other regions of the mammalian central nervous system.[6] DI $GABA_A$ receptors are characterized by low affinity of the prototypical 1,4-benzodiazepines (e.g., diazepam, flunitrazepam) which exhibit high affinity for other "diazepam-sensitive" (DS) $GABA_A$ receptors.[6–8] Molecular cloning and expression studies have demonstrated that a pharmacological profile similar to that of native DI $GABA_A$ receptors can be reconstituted in mammalian cells transfected with cDNA's encoding either α6, β2, and γ2 or α4, β2, and γ2 subunits.[9,10] The high affinity and selectivity of the putative ethanol antagonists Ro15-4513 (**20**), Ro15-3505(**22**), and Ro19-4603[7] for DI $GABA_A$ receptors, coupled with a low density of DI $GABA_A$ receptors in an "alcohol-nontolerant" rat,[11] and the cerebellar localization of this receptor isoform suggest the involvement of DI $GABA_A$ receptors in the motor incoordinating effects of ethanol. Several chemical families,[6,7,12,13] including imidazobenzodiazepines (e.g., Ro 15-4513, **20**), pyrazoloquinolinones (e.g., CGS 8216, **28**), and β-carbolines (e.g., DMCM) bind at DI $GABA_A$ receptors with high to moderate affinities. However, none of these compounds has shown enough selectivity at DI $GABA_A$ receptors compared to DS, which presents a significant obstacle to the characterization of the precise physiological and pharmacological role of this receptor isoform.

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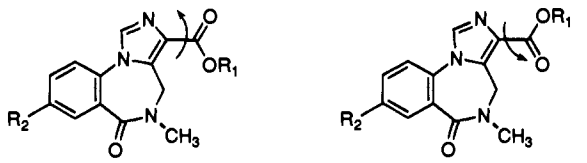


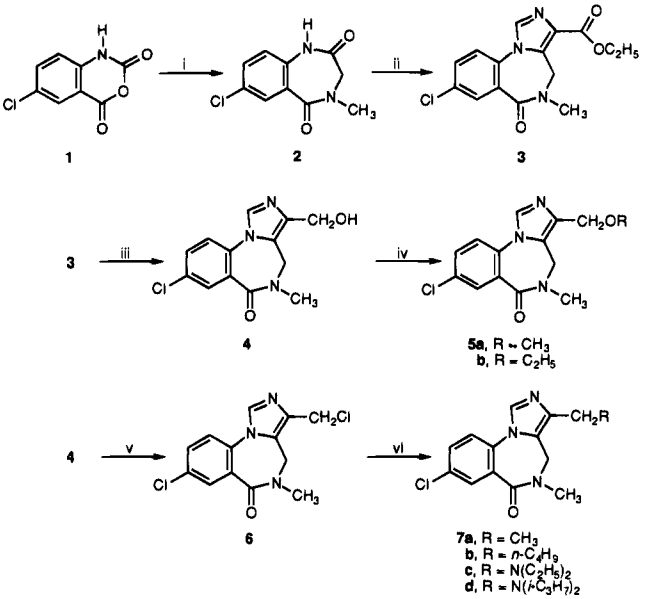
Figure 1. The *syn* and *anti* conformations of 3-alkyl ester-substituted imidazobenzodiazepines.

In order to understand the differences between the pharmacophores for the DI and the corresponding DS $GABA_A$ receptors, several structure–activity relationship studies (SAR) have been reported.[7,13–16] Data from these studies provide a basis upon which to search for even more selective ligands for DI $GABA_A$ receptors. Among the ligands studied to date, imidazo[1,5-*a*][1,4]-benzodiazepines are the most thoroughly investigated and exhibit the highest selectivity toward DI $GABA_A$ receptors. These studies[15,16] revealed that modifications to the 3-, 7-, and 8-positions of 6-oxoimidazo[1,5-*a*][1,4]-benzodiazepines have a marked influence on the affinity at DI relative to DS $GABA_A$ receptors. The substituent at position 3 of imidazobenzodiazepines is especially important for high potency and selectivity at DI $GABA_A$ receptors. However, previous SAR at the 3-position of these imidazobenzodiazepines was primarily confined to 3-alkyl ester congeners. In addition, the 3-alkyl ester can exist in two low-energy conformations (*syn* or *anti*), which makes it difficult to ascertain the precise structural requirements of the bioactive conformation at this position.[16] In the present study, the C(3) ester function of imidazobenzodiazepines related to Ro15-4513 (**20**) was replaced by other functional groups in order to better define the structural requirements for imidazobenzodiazepine binding at DS and DI $GABA_A$ receptors. These studies were intended to determine if the ester moiety of imidazobenzodiazepines was necessary for high affinity and selectivity at DI $GABA_A$ receptors, establish the conformation (*syn* or *anti*) at C(3) in the biologically active form (Figure 1), and facilitate the development of the pharmacophore at DI $GABA_A$ receptors. In addition, it had previously been noticed that the most active compounds at DI $GABA_A$ receptors were imidazobenzodiazepines which were substituted with an electron-withdrawing group at position 8. In order to determine the effect of electron-donating substituents at C(8) on DI $GABA_A$ receptor selectivity, novel imidazobenzodiazepines with alkoxy groups at C(8) were synthesized and evaluated at both DI and DS $GABA_A$ receptors.

Chemistry

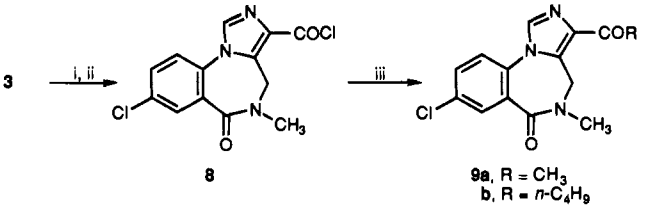
Since the 8-substituent of imidazobenzodiazepines was also important for potent ligand binding to the DI $GABA_A$ receptor, the new ligands reported here bear a chloro substituent at the 8-position and are derivatives of the most potent and selective DI $GABA_A$ receptor ligands including Ro15-1310, **3**. The starting imidazobenzodiazepine **3** (Ro15-1310) for this study was readily prepared from 5-chloroisatoic anhydride **1** as shown in Scheme 1, according to the method of Gu.[15] Selective reduction of the ester **3** at C(3) with lithium borohydride and methanol in a mixture of THF and diethyl ether provided the alcohol **4** which was subsequently converted into ethers **5a** and **5b** *via* a Williamson ether protocol (Scheme 1). Attempts to activate the hydroxyl group of alcohol **4** by tosylation (*p*-toluenesulfonyl chloride and pyridine) or bromination (dibromotriphenylphosphorane) failed to yield the desired derivatives. However, alcohol **4** could be quantitatively converted into the 3-(chloromethyl)imidazobenzodiazepine **6** on heating in a mixture of toluene and thionyl chloride. Treatment of **6** with alkylcopper lithium reagents or secondary amines afforded the desired congeners **7a**, **b**, **c**, and **d** (Scheme 1), respectively. The 3-alkylketo-substituted imidazobenzodiazepines **9a**, **b** were prepared by the reaction of various alkylcopper lithium reagents with acyl chloride **8**. The acyl chloride **8** had been obtained from hydrolysis of imidazobenzodiazepine **3**, followed by conversion of the 3-carboxylic acid function of the imidazobenzodiazepine into its acid chloride (Scheme 2).

Scheme 1[a]



[a] (i) CH_3NHCH_2COOH, DMSO, 150 °C; (ii) THF/DMF, NaH, $ClP(O)(OC_2H_5)_2$, 0 °C; THF/DMF, NaH, $CNCH_2COOC_2H_5$, 0 °C; (iii) $LiBH_4$–CH_3OH, THF/ethyl ether, reflux; (iv) DMSO, KOH, RI, 25 °C; (v) benzene, $SOCl_2$, reflux; (vi) THF, R_2CuLi, −78 °C or HNR'_2 (excess), room temperature or reflux.

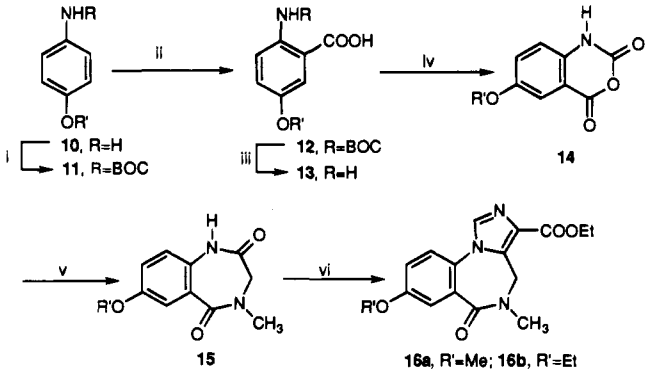
Scheme 2[a]



[a] (i) 10% KOH 25 °C; 1 N HCl; (ii) benzene, $SOCl_2$, reflux; (iii) THF, R_2CuLi, −78 °C.

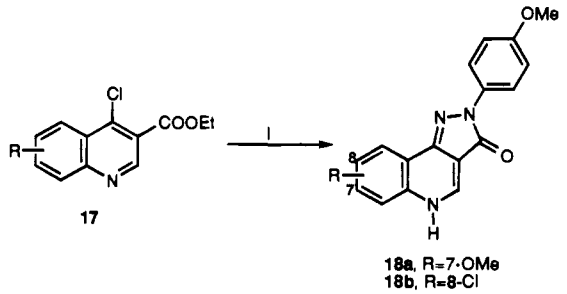
The synthesis of 8-alkoxyimidazobenzodiazepines is outlined in Scheme 3. A directed ortho-metalation strategy[17] was employed for the carbamate group exhibited a stronger directing influence than the alkoxy group. To our knowledge, this is the first time that this ortho-metalation strategy has been employed to prepare 8-alkoxyimidazobenzodiazepines. The BOC-protected 4-alkoxyanilines **11a** and **11b** were selectively metalated with *t*-BuLi at the ortho position of the carbamate

Scheme 3[a]



[a] (i) THF, $(BOC)_2O$, reflux, 98%; (ii) THF, *t*-BuLi, −78 °C; THF, CO_2, −78 °C; 1 N HCl, 80%; (iii) concentrated aqueous HCl, THF, 25 °C, pH = 4.5, 80%; (iv) 1 N HCl, ClCOCl, 25 °C, 95%; (v) CH_3NHCH_2COOH, DMSO, 150 °C, 90%; (vi) NaH, THF/DMF, $ClP(O)(OEt)_2$, 0 °C; NaH, THF/DMF, $CNCH_2COOEt$, 0 °C, 50–60%.

Scheme 4[a]



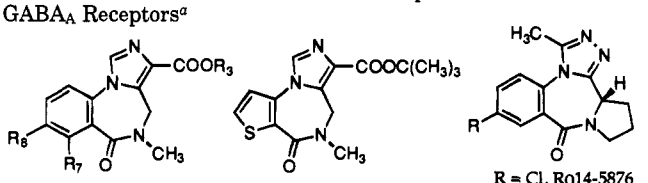
[a] (i) (4-Methoxyphenyl)hydrazine, Dowtherm A, 180 °C.

group to give, after carboxylation and an acid-catalyzed hydrolysis, the aryl amino acids **13a** and **13b**, respectively. Treatment of amino acids **13a** and **13b** with phosgene in 1 N HCl afforded isatoic anhydrides **14a** and **14b**, respectively, which were used to prepare the corresponding imidazobenzodiazepines **16a** and **16b**, as described above. The new pyrazoloquinolinone derivatives **18a** and **18b** were prepared according to the procedure reported in literature (Scheme 4).[18]

Results and Discussion

Imidazobenzodiazepines are well-documented to exhibit high potency and a wide spectrum of *in vivo* activity at DS $GABA_A$ receptors.[19] Recently, these compounds have also been described as high affinity ligands at DI $GABA_A$ receptors (Table 1).[7,9] Subsequently, SAR studies revealed that the substituents at the C(3) and C(8) positions are critical for high affinity and selectivity for DI $GABA_A$ receptors.[15,16] More specifically, *in vitro* binding studies demonstrated that a proper substituent (e.g., an alkyl ester group) with a preferred size at the C(3) position of imidazobenzodiazepines was required.[15,16] This finding indicated that a lipophilic pocket within the DI $GABA_A$ receptors corresponding to the C(3) position of imidazobenzodiazepines might be present to interact with this lipophilic group. While the prototypical substituent at the 3-position of the imidazo[1,5-*a*][1,4]benzodiazepines (e.g., Ro15-4513, **20**) was an ester group, this substituent can exist in either of two low-energy conformations (*syn* or *anti*) which makes determination of the bioactive conformation at DI $GABA_A$ receptors problematic. The size

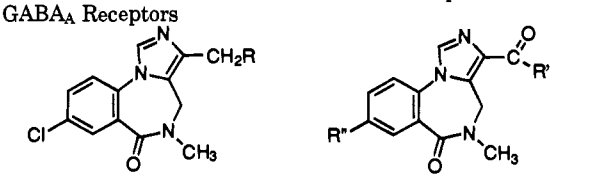
Table 1. Affinities of Imidazobenzodiazepines at DS and DI $GABA_A$ Receptors[a]



compd	R_3	R_7	R_8	DS (nM)	DI (nM)
3	CH_2CH_3 (Ro15-1310)		Cl	5.4 ± 0.9	16.9 ± 1.1
19	CH_3		Cl	29.8 ± 5.4	123.5 ± 1.8
20	CH_2CH_3 (Ro15-4513)		N_3	5.3 ± 1.2	3.1 ± 0.1
21	CH_2CH_3 (Ro14-7437)		H	1.3 ± 0.1	214 ± 4.0
22	CH_2CH_3 (Ro15-3505)	Cl		0.2 ± 0.0	20 ± 2.4
23	CH_2CH_3 (Ro15-1788)		F	0.8 ± 0.1	58 ± 11
24	$CH_2CH_2CH_3$		Cl	24.8 ± 1.4	33.3 ± 2.3
25	$C(CH_3)_3$		Cl	4.0 ± 0.9	1.7 ± 0.3
26	$CH(CH_2CH_3)_2$		Cl	26.9 ± 9.2	122.6 ± 11.7
27	$CH_2CH_2C(CH_3)_3$		Cl	184 ± 1.4	>1000
Ro19-4603				0.2 ± 0.0	2.6 ± 0.2
Ro14-5876				inactive[b]	
Ro14-7059				inactive[b]	

[a] K_i values from refs 15 and 16. [b] See ref 19.

Table 2. Affinities of Novel Imidazobenzodiazepines at DS and DI $GABA_A$ Receptors



compd	R	R′	R″	DS (nM)[a]	DI (nM)
4	OH			>1000 (>1000)	>1000
5a	OCH_3			514 ± 34 (>1000)	>1000
5b	OC_2H_5			>500 (>1000)	>1000
6	Cl			>500 (540 ± 45)	>1000
7a	CH_3			>500 (778 ± 31)	>1000
7b	*n*-C_4H_9			>1000 (>1000)	>1000
7c	$N(C_2H_5)_2$			>1000 (>1000)	>1000
7d	$N[CH(CH_3)_2]_2$			>1000 (>1000)	>1000
9a		CH_3	Cl	>1000 (>1000)	>1000
9b		*n*-C_4H_9	Cl	>500 (>1000)	>1000
16a		OEt	OMe	2.47 ± 0.19	23.8 ± 3.8
16b		OEt	OEt	7.07 ± 0.5	40.9 ± 4.6

[a] Reported as IC_{50} values. The data in parentheses are K_i values obtained from cortical membranes using the methods described in ref 21.

of this ester group was well-defined[15] (ethyl to *tert*-butyl ester) for high-affinity binding to DI $GABA_A$ receptors (Table 1), but the role of the two oxygen atoms on the ester function was not defined. Moreover, although examination of the CoMFA results[16] suggested that the bioactive conformation of the ester was *anti* in the Ro15-4513 (**20**) series, confirmation of this hypothesis had not been addressed. Since imidazobenzodiazepines are, to date, the most potent and selective DI $GABA_A$ receptor ligands, the relationship between the pharmacophores at both DI and DS sites becomes important in the search for novel potent selective ligands for DI $GABA_A$ receptors.

Examination of the affinities of these novel imidazobenzodiazepines (Table 2) revealed that the oxygen atoms on the ester group are essential for high affinity for both DS and DI $GABA_A$ receptors and reemphasized the importance of the 3-alkyl ester group.[7,14–16] This finding was supported by the *in vitro* binding data of the 3-alkyl- and 3-dialkylamino-substituted analogs **7a**, **7b**, **7c**, and **7d** (**7a**, >500 nM; **7b**, **7c**, **7d**, >1000 nM) which lacked high affinity for either DI or DS $GABA_A$

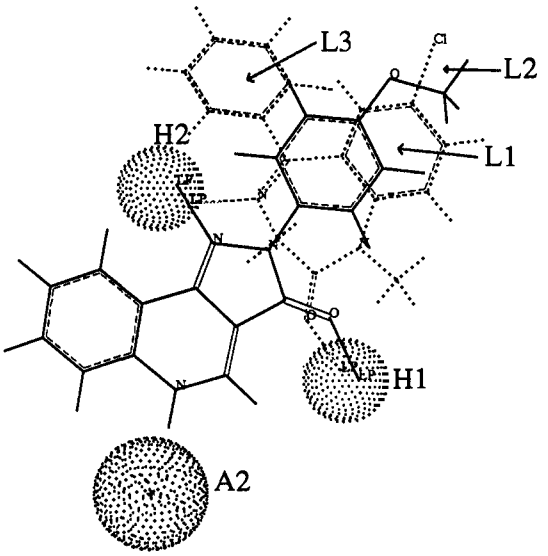


Figure 2. The pharmacophore of DS $GABA_A$ receptor ligands: the overlap of diazepam (dashed line) and pyrazoloquinolinone **30** (solid line). (a) The agonist pharmacophore is characterized by H1, H2, L1, L2, and L3; (b) the inverse agonist pharmacophore requires A2, H1, and L1 for potent inverse agonist activity.

receptors. The low affinities of these 3-alkyl-substituted analogs may be attributable to the absence of the oxygen atoms of the ester function since the 3-*n*-butyl substituent at C(3) (see **7b**) has the correct size required for imidazobenzodiazepine binding to $GABA_A$ receptors. Nonetheless, this agent exhibited low affinity at both DI and DS $GABA_A$ receptors (>1000 nM). The low affinity at DS $GABA_A$ receptors, which was also due to the absence of a 3-substituted ester moiety, has also been noted in Ro14-5876 and Ro14-7059 (Table 1).[19] These results strongly suggest that the oxygen atoms on the 3-alkyl ester group are important for high-affinity binding of imidazobenzodiazepine ligands at $GABA_A$ receptors. One of these oxygen atoms may be involved in the formation of an additional hydrogen bond (at H2) as a hydrogen bond acceptor site on the ligand. The requirement of an additional hydrogen bond (H2 as shown in Figure 2) *via* the 3-alkyl ester substituent at the DS $GABA_A$ receptors is consistent with the DS pharmacophore developed in this laboratory (Figure 2).[16,20] This pharmacophore was established based on ligands other than the series related to Ro15-4513, **20**.[21]

It is well-recognized via ligand stereoselectivity[22] and molecular modeling[20] that the pharmacophoric descriptors for DI and DS binding sites are similar and that the size and location of lipophilic pockets constitute a major difference between DS and DI receptor subsites.[23] The common structural elements of the DS pharmacophore represented by L1 and H1 (Figure 2) appear to be the same for the DI site (L1 and H1, as shown in Figure 3a). In addition to these common elements (L1 and H1), the *in vitro* data of the 3-alkyl substituted analogs reported here suggested that formation of a second hydrogen bond using one of the 3-alkyl ester oxygen atoms as a hydrogen bond acceptor group was also important for high-affinity binding of imidazobenzodiazepine ligands to DI $GABA_A$ receptors. Nevertheless, the alignment of the second hydrogen bond (labeled as H2) between the oxygen atom on the 3-substituted ester group and the active proton on the DI $GABA_A$

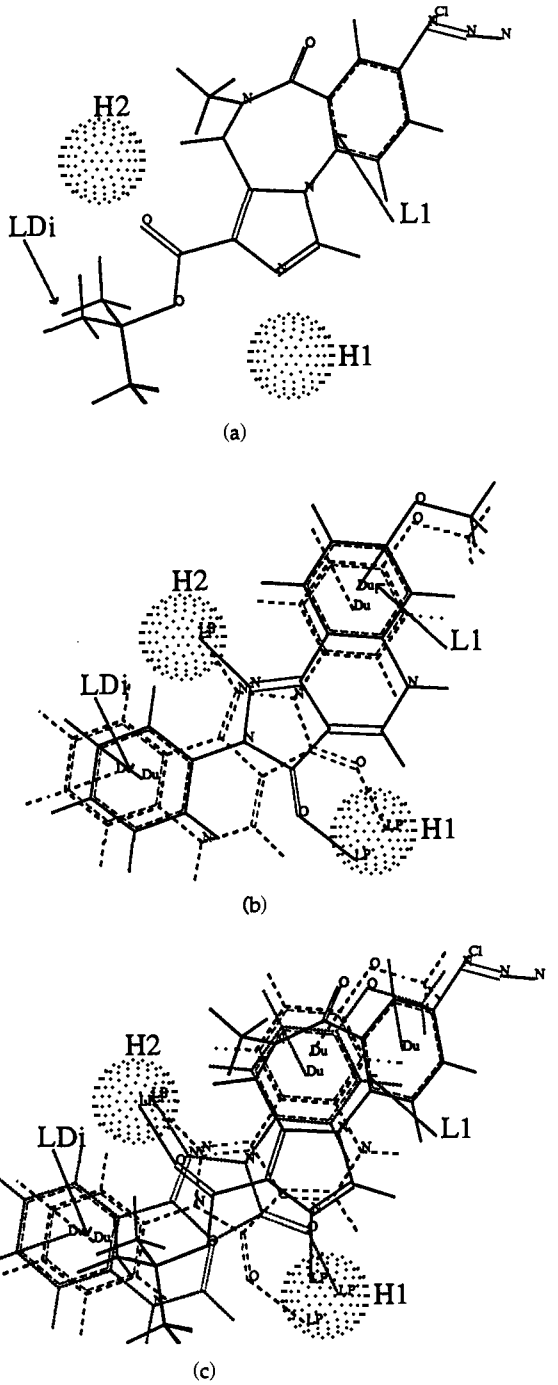
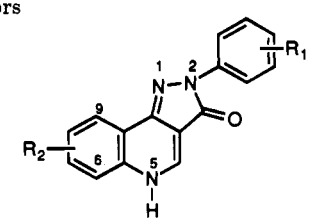


Figure 3. The proposed pharmacophore of DI $GABA_A$ receptor ligands consisting of two hydrogen bond acceptor sites (H1 and H2) and two lipophilic pockets (L1 and LDi): (a) the overlap of imidazobenzodiazepines Ro15-4513 (**20**) and **25**; (b) the overlap of pyrazolo[4,3-c]quinolinones **30** (solid line) and **31** (dashed line); (c) the overlap of imidazobenzodiazepines Ro15-4513 (**20**) and **25** (solid line) and pyrazolo[4,3-c]quinolinones **30** (dashed line) and **31** (dotted line).

receptor had not been defined previous to this work. A rather small database of DI $GABA_A$ receptor ligands as well as a limited number of structural variations has impeded the understanding of the pharmacophore for this site. To date, the pyrazoloquinolinones (Table 3)[13] are the only other group of compounds with potency comparable to the imidazobenzodiazepines at the DI site. Because the imidazobenzodiazepines and pyrazoloquinolinones both exhibit high affinities at DI $GABA_A$ receptors, the pharmacophores of these two

Table 3. Affinites of Pyrazolo[4,3-c]quinolinones at DI and DS $GABA_A$ Receptors



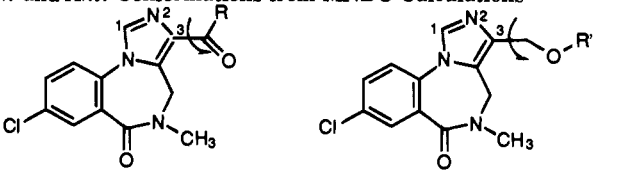
compd	R_1	R_2	DS (nM)	DI (nM)
18a	*p*-OCH_3	7-OCH_3	0.40 ± 0.02	10 ± 2.1
18b	*p*-OCH_3	8-Cl	0.23 ± 0.03	1.9 ± 0.2
28[a]	H	H	0.13 ± 0.01	46 ± 15
29[a]	*m*-OCH_3	H	0.12 ± 0.01	26 ± 1.6
30[a]	*p*-OCH_3	H	0.21 ± 0.00	9.3 ± 0.2
31[a]	H	7-OCH_3	0.88 ± 0.03	1.9 ± 0.2
32[a]	H	8-OCH_3	0.09 ± 0.00	47 ± 2.2
33[a]	H	8-Cl	0.05 ± 0.00	5.1 ± 0.3

[a] K_i values from ref 13.

series of ligands at the DI site must be superimposable. Consequently, the requirement of two hydrogen bond acceptor atoms for the imidazobenzodiazepines at DI $GABA_A$ receptors, coupled with the alignment of two possible hydrogen bond acceptor groups on the pyrazoloquinolinones (Figure 3b), clearly suggested an alignment of the imidazobenzodiazepines, as shown in Figure 3a. Such an alignment ensures the superposition of pharmacophores from these two types of high-affinity ligands at the DI site (Figure 3c). These results are consistent with the CoMFA analysis[16] of 3-alkyl ester-substituted imidazobenzodiazepines (Ro15-4513, **20** series), which predicted that a more negative electrostatic interaction at the *anti* position of the imidazole N(2) nitrogen atom would increase the potency of imidazobenzodiazepine binding to DI $GABA_A$ receptors.

The proposed pharmacophore can be employed to explain the low potency of 3-alkylketo- and 3-alkyl ether-substituted analogs (**5a**, **5b**, **9a**, and **9b**) (Figure 3a–c). The low affinities of these analogs at $GABA_A$ receptors (Table 2) are due to unfavorable interactions of these ligands with the $GABA_A$ receptor protein. Using quantum mechanics (see molecular modeling section for details), the relative stabilities in terms of the heat of formation of *syn* and *anti* conformations of these compounds were calculated and compared to those of the 3-alkyl ester-substituted analogs (Table 4). The *syn* conformation of the 3-alkylketo analog (**9b**) was not a favorable conformation based on its heat of formation (−19.1997 kcal/mol for the *syn* compared to −24.5657 kcal/mol for the *anti* isomer). Thus, the *syn* conformation was not believed to be the active one at either DI or DS sites. The superposition of 3-alkyl ester- and 3-alkylketo-substituted imidazobenzodiazepines in their *anti* conformation (Figure 4) revealed that both series of ligands possessed very similar topographic features and were capable of formation of a hydrogen bond at position H2. However, close examination of the *anti* conformation of the 3-alkylketo function demonstrated that a strong steric repulsion (Figure 4) at position H1 existed and would interrupt the required hydrogen bond formation at that descriptor. This negative interaction interferes with the binding of the 3-alkylketo-substituted imidazobenzodiazepines to both the DI and DS sites. This result is in agreement with the CoMFA analysis,[16] which demonstrated that a low steric interaction adjacent to the imidazole N(2) position (corresponding to the position of an α-methylene group) would increase affinity at DI $GABA_A$ receptors. On the other hand, the 3-alkyl ester group in its *anti* conformation has an electronegative oxygen atom in place of the methylene unit and, therefore, will enhance the stability of the hydrogen bond at H1. This results in higher affinity at both the DI and DS sites for the parent (Ro15-4513, **20**) series.

Table 4. Heats of Formation of 3-Alkyl Ester-, 3-Alkylketo-, and 3-Alkyl Ether-Substituted Imidazobenzodiazepines in Their *Syn* and *Anti* Conformations from MNDO Calculations



compd	R	R′	$N_2-C_3-C{=}O$ or N_2-C_3-C-O torsion angle (deg)[a]	heat of formation (kcal/mol)
24 (*syn*)	OC_3H_7		22.0	−61.2430
24 (*anti*)	OC_3H_7		154.9	−62.9773
5b (*syn*)		C_2H_5	19.0	−20.7036
5b (*anti*)		C_2H_5	179.5	−21.6424
9b (*syn*)	C_4H_9		10.2	−19.1997
9b (*anti*)	C_4H_9		170.6	−24.5657

[a] When N_2 and O of the C=O or CH_2OR' lie in the opposite directions, the angle is defined as 180°.

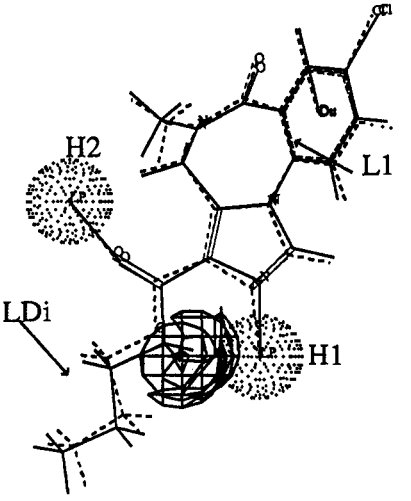


Figure 4. The overlap of 3-alkylketo-substituted imidazobenzodiazepine **7b** (solid line) and 3-alkyl ester-substituted imidazobenzodiazepine **24** in their *anti* conformation. The prohibited repulsion is shown by the negative interaction of van der Waals volume (solid line contour) of the methylene group from the ketone and H1.

As indicated in Table 4, the geometry optimization of 3-alkyl ether-substituted imidazobenzodiazepines indicates that both *syn* and *anti* conformations are low in energy and are thus potential bioactive conformations. However, the *syn* conformation of these compounds as shown is not an active conformation due to the absence of an interaction at H2 (Figure 5a). While the *anti* conformation appears to contain hydrogen bond acceptor groups available to interact with $GABA_A$ receptors at both H1 and H2 (Figure 5b), these 3-alkyl ether-substituted imidazobenzodiazepine analogs exhibit low affinities at DI and DS $GABA_A$ receptors. This result seems to suggest that the conformation in Figure 5b is an unfavorable conformation for high-affinity binding to $GABA_A$ receptors. The *anti* conformation of the

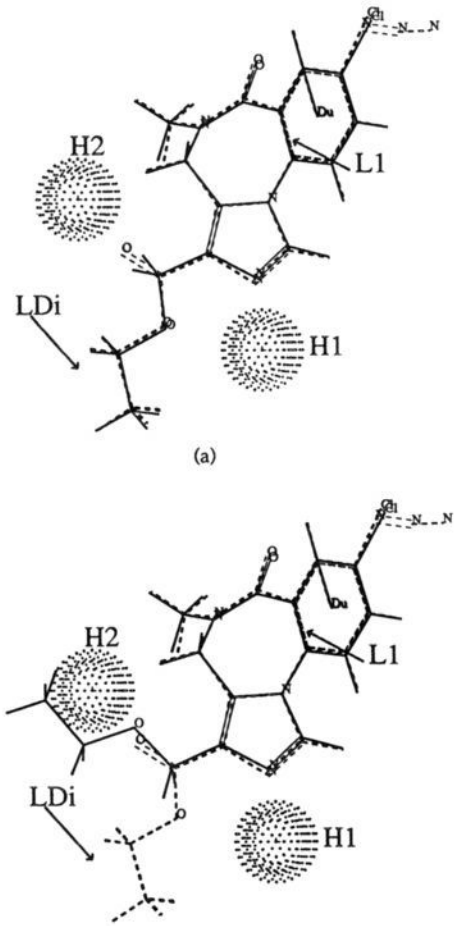


Figure 5. The overlap of (a) 3-alkyl ether-substituted imidazobenzodiazepine **5b** (solid line, *syn*) with Ro15-4513 (**20**) (dashed line, *anti*) and (b) 3-alkyl ether-substituted imidazobenzodiazepine **5b** (solid line, *anti*) with Ro15-4513 (**20**) (dashed line, *anti*).

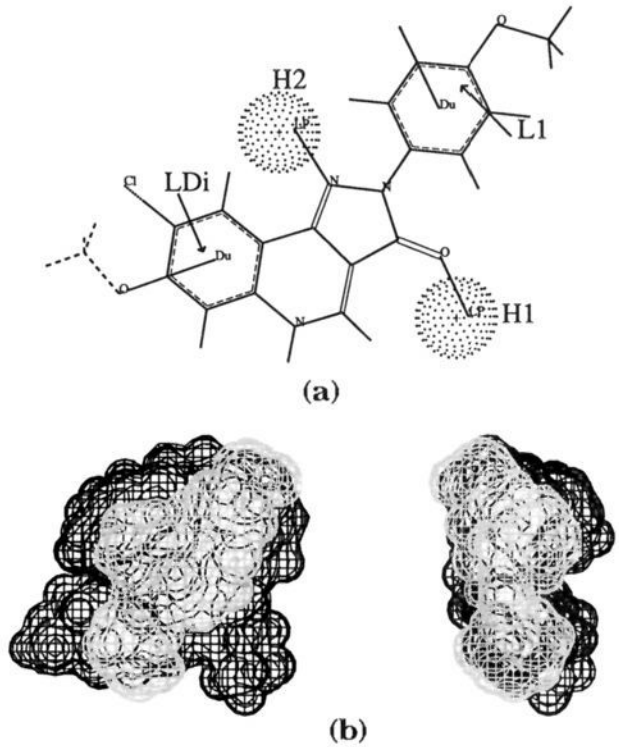


Figure 6. (a) The overlap of three pyrazoloquinolinones **18a** (dashed line), **18b** (dotted line), and **30** (solid line). (b) The overlap of DS (dark line, the union of the volumes of 36 agonist ligands from more than 10 different chemical families[23]) and DI (light line, the union of volumes of DI ligands **16a**, **16b**, **19**, **20**, **21**, **22**, **23**, **24**, **25**, and **26**) included volumes. The figure on the right is simply rotation of the figure on the left by 90° in a clockwise direction.

3-alkyl ether-substituted imidazobenzodiazepines in Figure 5b also shows an unfavorable steric repulsion between the 3-alkyl ether group and H2, which may explain the low affinities of the new ether congeners (**5a**,**b**). This result is in agreement with the previous CoMFA results[16] and indicates that the active conformation of the potent 3-alkyl ester ligands in the parent (Ro15-4513, **20**) series should be *anti* at both DS and DI $GABA_A$ receptors. The bioactive *anti* conformation proposed previously by modeling from this laboratory[16] and supported in the present study for the DS pharmacophore is in contrast to the *syn* conformation reported in the literature.[24,25] Clearly, the *syn* conformation would exhibit an unfavorable interaction with the DS $GABA_A$ receptors at descriptor H2.

Examination of the *in vitro* binding data of new 8-alkoxyimidazobenzodiazepine derivatives (**16a** and **16b**) indicated that an electron-donating substituent at the C(8) position increased the affinity of imidazobenzodiazepines for the DI site and decreased the affinity for the DS site compared to the unsubstituted analog (for example, 8-methoxyimidazobenzodiazepine **16a** was 8-fold more potent than the 8-unsubstituted analog **21** at the DI site, IC_{50} (nM) 23.8 vs 214; however, **16a** was less potent than **21** at the DS site, IC_{50} (nM) 2.47 vs 1.3). Nevertheless, affinities of 8-alkoxy-substituted analogs were very similar to those of imidazobenzodiazepines which have an electron-withdrawing group at the C(8) position for both the DI and DS sites [e.g. 8-methoxy- (**16a**) and 8-chloro- (**3**) substituted imidazobenzodiazepines have similar IC_{50}s (nM) at the DI site (23.8 vs 16.9) and at the DS site (2.4 vs 5.4)]. This finding revealed that the electron-withdrawing and -donating functions at the 8-position of imidazobenzodiazepines have a similar effect on ligand affinity and selectivity for both the DI and DS sites.

The design and synthesis of the two pyrazoloquinolinone derivatives were intended to confirm the lipophilic interactions at LDi proposed in the pharmacophore. It was anticipated that an increase in the lipophilicity of ligands at the LDi region would improve the affinity of ligands for DI but not for DS $GABA_A$ receptors, while a decrease of the lipophilicity of ligands in the same region would diminish the affinity of ligands at DI $GABA_A$ receptors. In this regard, a chlorine atom (lipophilic, π value approximately 0.70[26]) and a methoxy group (slightly hydrophilic, π value approximately −0.02[26]) as a lipophilic and a weak hydrophilic factor, respectively, were employed to alter the relative regional lipophilicity of ligands at LDi. Thus, ligand **18a** which has a methoxy group, and very similar lipophilicity at LDi (Figure 6a) compared to parent compound **30** would, therefore, exhibit similar affinity at DI $GABA_A$ receptors, while ligand **18b** which has a chlorine substituent and thus higher lipophilicity at LDi (Figure 6) relative to ligand **30** should increase the binding affinity at DI $GABA_A$ receptors. As demonstrated in Table 3, ligands

18a and **30** have similar affinities at DI $GABA_A$ receptors (10 vs 9.3), while ligand **18b** has higher affinity at DI $GABA_A$ receptors than ligand **30** does (1.9 vs 9.3). This result suggests the importance of lipophilic interactions between the ligands and DI $GABA_A$ receptors at the LDi region and supports the pharmacophore proposed above.

In summary, the systematic variation of substituents at the C(3) position of the imidazobenzodiazepines revealed the importance of the oxygen atoms on the ester group for receptor binding at DI $GABA_A$ receptors in the parent (Ro15-4513, **20**) series. The formation of the secondary hydrogen bond at H2 using one of the oxygen atoms on the ester group seems to be a common feature of imidazobenzodiazepines which interact at DS and DI $GABA_A$ receptors. In addition, the establishment of the bioactive *anti* conformation of the 3-alkyl ester group of imidazobenzodiazepines based on the binding data of the new imidazobenzodiazepine analogs further defines the structural preference at C(3) and supports the CoMFA results reported earlier from this laboratory.[16] Accordingly, a DI $GABA_A$ receptor pharmacophore (Figures 3a–c) was proposed based upon the SAR of these new imidazobenzodiazepine analogs and on several pyrazoloquinolinones. Examination of this model suggests that the DI $GABA_A$ receptor pharmacophore preserves the common structural elements (L1, H1, and H2) of the DS pharmacophore, but some of the lipophilic pockets are different in size. The absence of lipophilic pocket L3 in the DI $GABA_A$ receptor pharmacophore vs its presence in the DS sites is important in this regard. On the basis of the activity of ligands described here when combined with previous work,[16,20,23] it is clear that the DI site is a subsite of the DS site, and this is clearly illustrated in the overlap of the included volumes of these two sites in Figure 6b. Ligands which carry large substituents that occupy L3 will clearly not bind to the smaller DI site. For potent affinity at the DI site, the imidazobenzodiazepines require the presence of a lipophilic substituent (ethyl, *tert*-butyl ester) in the pocket designated LDi, as illustrated in Figure 3, which is not required for ligands with potent affinity at DS sites (for example, methyl ester **19**). Current efforts are in progress to use this pharmacophore to direct the design and synthesis of ligands more selective for the DI site over the DS sites.

Experimental Section

Radioligand Binding. [^{3}H]Ro15-4513 binding to DI and DS $GABA_A$ receptors in rat cerebellum was determined as previously described.[13] In brief, cerebella from adult, male Sprague–Dawley rats were dissected, weighed, and disrupted (Brinkmann Polytron, setting 6, 10 s) in 60 volumes of 50 mM Tris citrate buffer (pH = 7.8). Homogenates were centrifuged at 20000*g* for 20 min (4 °C), resuspended in 50 volumes of buffer, and recentrifuged. This centrifugation procedure was repeated five times. Membranes were reconstituted in 50 volumes of buffer and stored frozen at −70 °C prior to use. Radioligand binding to DS + DI $GABA_A$ receptors was determined in a solution consisting of: tissue (~100 μg), [^{3}H]-Ro15-4513 (Sp. Act. 24.3 Ci/mmol, New England Nuclear, final concentration ~2 nM), 0.2 M NaCl, drug, Tris citrate buffer (pH = 7.8) to a total volume of 0.5 mL. Radioligand binding to DI $GABA_A$ receptors was determined exactly as described above except that 10 μM of diazepam was also added to the solution; binding to DS was the difference between values obtained for ligand binding to DI and DS + DI $GABA_A$ receptors. Nonspecific binding was determined in the presence of 10 μM Ro15-1788 and was typically <10% and <20% of total binding for DS and DI $GABA_A$ receptors, respectively. Assays were performed in duplicate. Values are the mean ± standard error unless otherwise indicated. Incubations were terminated after 60 min by rapid filtration through Whatman GF/B filters with a Brandel (M48-R) manifold followed by two 5 mL washes with ice-cold buffer. The radioactivity retained on the filter was measured by scintillation counting. IC_{50} values were generated using at least six concentrations of compound with an iterative curve fitting program (Graph Pad, Inplot 4.0).

Molecular Modeling. Sybyl (version 5.5, Tripos Associates, St. Louis, MO) calculations were performed on a Silicon Graphics Personal Iris 4D/35. The starting geometry of the 6-oxoimidazo[1,5-*a*][1,4]benzodiazepine cores (Table 3) were constructed from *ab initio*-optimized geometry.[21] Substituent groups at C(3) were then added to the parent compounds to generate the novel analogs using Sybyl. The geometry of flexible side chains and hydrogen atoms of novel imidazobenzodiazepine analogs were minimized using a MNDO program (within MOPAC version 5.00, using SCF_CONVERGENCE-_CRITERION option with criterion set at 0.000 01) in Sybyl holding the heterocyclic core fixed. The calculations of low-energy conformation and its corresponding energy (heat of formation) were carried out using MNDO full geometry optimization option in the local region. The lengths of hydrogen bond extension vectors (HBV) were set to 1.84 Å, while the C–N–HBV and C=O–HBV valence angles used were set to 120 and 135°, respectively, to mimic the geometry of an ideal hydrogen bond.[21,27–34]

Materials. 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 2-amino-5-methoxybenzoic acid was purchased from Lancaster Synthesis Inc.

8-Chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-methyl Alcohol (4). A solution of imidazobenzodiazepine **3**[15] (5 g, 15.6 mmol) in a mixture of ethyl ether (50 mL), anhydrous CH_3OH (2.5 mL), and THF (50 mL) was treated with $LiBH_4$ (2.0 M in THF, 9 mL, 18 mmol). The mixture which resulted was heated to reflux for 30 min, cooled to room temperature, and treated with saturated aqueous $NaHCO_3$ (5 mL). The solvent was then removed under reduced pressure, and the residue was taken up in EtOAc (100 mL). The organic layer was washed with water (2 × 20 mL) and brine (20 mL) and dried ($MgSO_4$). After removal of solvent under reduced pressure, the residue was purified by flash chromatography (silica gel, EtOAc) to afford alcohol **4** as colorless crystals (2.9 g, 67%): mp 252–253 °C; IR (KBr) 3500 (br, OH), 3100, 1667, 1612, 823 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.20 (s, 3H), 4.40 (s, 2H), 4.70 (d, 2H, J = 4.2 Hz), 7.30 (d, 1H, J = 8.6 Hz), 7.55 (dd, 1H, J = 8.7, 2.4 Hz), 7.80 (s, 1H), 8.00 (d, 1H, J = 2.4 Hz); MS (EI) m/e 279 (M^+, 41), 277 (M^+, 100), 259 (84), 246 (55), 231 (41). Anal. ($C_{13}H_{12}ClN_3O_2 \cdot {}^1/_4H_2O$) C, H, N.

Methyl (8-Chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepin-3-yl)methyl ether (5a). To a slurry of KOH (100 mg, 1.6 mmol) in DMSO (2 mL) at room temperature were added alcohol **4** (108 mg, 0.4 mmol) and CH_3I (50 mL, 0.8 mmol). The mixture which resulted was stirred for 5 min, poured into ice water (10 mL), and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine (10 mL) and dried ($MgSO_4$). After removal of solvent under reduced pressure, the residue was purified by a wash column on silica gel (EtOAc) to give ether **5a** as an

off-white powder (110 mg, 95%): mp 193–194 °C; IR (KBr) 3122, 2973, 1632, 1611, 811 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.18 (s, 3H), 3.42 (s, 3H), 4.38 (s, 2H), 4.55 (br, 2H), 7.30 (d, 1H, J = 8.6 Hz), 7.55 (dd, 1H, J = 8.7, 2.5 Hz), 7.80 (s, 1H), 8.00 (d, 1H, J = 2.4 Hz). Anal. ($C_{14}H_{14}ClN_3O_2$) C, H, N.

Ethyl (8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepin-3-yl)methyl ether (5b) was prepared from alcohol **4** and C_2H_5I using the procedure as described for the preparation of ether **5a**. Purification by flash chromatography (silica gel, EtOAc) gave ether **5b** in 93% yield as white crystals: mp 137–138 °C; IR (KBr) 3182, 2973, 1634, 1601, 810 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.22 (t, 3H, J = 7.0 Hz), 3.12 (s, 3H), 3.60 (q, 2H, J = 7.0 Hz), 4.35 (br, 2H), 4.55 (s, 2H), 7.30 (d, 1H, J = 8.6 Hz), 7.50 (dd, 1H, J = 8.5, 2.4 Hz), 7.86 (s, 1H), 7.92 (d, 1H, J = 2.4 Hz). Anal. ($C_{15}H_{16}ClN_3O_2 \cdot {}^1/_3H_2O$) C, H, N.

3-(Chloromethyl)-8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (6). A mixture of alcohol **4** (0.43 g, 1.55 mmol), thionyl chloride (6 mL), and anhydrous toluene (50 mL) was heated to reflux for 3 h. The solvent was removed under reduced pressure to afford **6** as a yellow colored solid. Recrystallization from a mixture of EtOAc and CH_3CH_2OH (1:1) gave **6** as white crystals: mp 274–294 °C dec; IR (KBr) 3144, 2876, 1643, 1602, 786 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.20 (s, 3H), 4.40 (s, 2H), 4.70 (s, 2H), 7.40 (d, 1H, J = 8.6 Hz), 7.60 (dd, 1H, J = 8.6, 2.4 Hz), 8.00 (s, 1H), 8.05 (d, 1H, J = 2.4 Hz); MS (EI) m/e 298 (M^+, 70), 296 (M^+, 100), 262 (35), 260 (70). Anal. ($C_{13}H_{11}Cl_2N_3O \cdot {}^1/_3H_2O$) C, H, N.

8-Chloro-5,6-dihydro-3-ethyl-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (7a). A slurry of CuSCN (122 mg, 1.0 mmol) in THF (10 mL) under argon at −15 °C was treated with methyllithium in an etheral solution (1.4 M, 1.42 mL, 2.0 mmol). The mixture which resulted was stirred for 30 min at −15 °C, slowly warmed to 0 °C to give a clear solution, and then introduced to a solution of **6** (100 mg, 0.32 mmol) in THF (5 mL) under a positive pressure of argon at 0 °C. After 2 h, the reaction mixture was brought to room temperature, treated with saturated aqueous $NaHCO_3$ (5 mL), and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic extracts were washed with water (2 × 20 mL) and brine (10 mL) and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (silica gel, EtOAc) to give **7a** (49 mg, 56%) as a white powder: mp 189–191 °C; IR (KBr) 3120, 2897, 1632, 1601, 801 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (t, 3H, J = 7.6 Hz), 2.65 (q, 2H, J = 7.5 Hz), 3.20 (s, 3H), 4.30 (br, 2H), 7.32 (d, 1H, J = 8.5 Hz), 7.55 (dd, 1H, J = 8.6, 2.4 Hz), 7.81 (s, 1H), 8.02 (d, 2.4 Hz); MS (EI) m/e 277 (M^+, 32), 275 (M^+, 100), 246 (35), 219 (62). Anal. ($C_{14}H_{14}ClN_3O$) C, H, N.

8-Chloro-5,6-dihydro-5-methyl-3-pentyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (7b). Benzodiazepine **7b** was prepared as an oil in 54% yield from **6** and *n*-butyllithium using the procedure described for the preparation of **7a**. Recrystallization of the free base of benzodiazepine **7b** from ethanolic hydrogen chloride solution afforded the hydrochloride salt of **7b** as green crystals: mp 207–210 °C; IR (KBr), 3211, 3102, 2876, 1632, 1601, 827 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (br, 3H), 1.35 (br, 3H), 1.65 (br, 3H), 2.61 (br, 2H), 3.15 (s, 3H), 4.11–4.52 (br, 2H), 7.30 (d, 1H, J = 8.3 Hz), 7.55 (dd, 1H, J = 8.4, 2.4 Hz), 7.81 (br, 1H), 8.02 (d, 1H, J = 2.3 Hz); MS (EI) m/e 319 (M^+, 9.9), 317 (M^+, 28), 276 (10), 274 (31), 263 (33), 261 (100), 234 (11), 232 (30), 221 (10), 219 (34). Anal. ($C_{17}H_{20}ClN_3O$) C, H, N.

3-[(*N*,*N*-Diethylamino)methyl]-8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (7c). A mixture of **6** (0.176 g, 0.6 mmol), diethylamine (5 mL), and THF (10 mL) was stirred overnight. The solvent and excess diethylamine were removed under reduced pressure. The residue was taken up in EtOAc (50 mL), washed with water (2 × 10 mL) and brine (10 mL), and dried ($MgSO_4$). After removal of solvent under reduced pressure, a brown oil was obtained. Recrystallization of the oil from EtOAc afforded benzodiazepine **7c** as white crystals (0.195 g, 97%): mp 145–146 °C; IR (KBr) 3108, 2903, 1638, 1601, 794 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.05 (t, 6H, J = 7.1 Hz), 2.55 (q, 4H, J = 7.1 Hz), 3.22 (s, 3H), 3.60 (s, 2H), 4.31–4.60 (br, 2H), 7.30 (d, 1H, J = 8.5 Hz), 7.51 (dd, 1H, J = 8.0, 2.6 Hz), 7.73 (s, 1H), 7.98 (d, 1H, J = 2.0 Hz); MS (EI) m/e 334 (M^+, 1.3), 332 (M^+, 3.5), 263 (33), 261 (100), 207 (9), 205 (27). Anal. ($C_{17}H_{21}ClN_4O$) C, H, N.

3-[(*N*,*N*-Diisopropylamino)methyl]-8-chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (7d). A mixture of benzodiazepine **6** (136 mg, 0.46 mmol), diisopropylamine (5 mL), and THF (10 mL) was heated to reflux for 3 days. The solvent and excess diisopropylamine were removed under reduced pressure, and the residue was taken up in EtOAc (30 mL), washed with saturated aqueous $NaHCO_3$ (10 mL), water (2 × 10 mL), brine (10 mL), and dried ($MgSO_4$). After removal of solvent under reduced pressure, the residue was purified by a wash column on aluminum oxide to give **7d** as a white solid (140 mg, 85%): 199–201 °C; IR (KBr) 3123, 2913, 1634, 1607, 800 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.11 (d, 12H, J = 6.7 Hz), 3.12 (m, 2H), 3.20 (s, 3H), 3.78 (s, 2H), 4.41 (s, 1H), 4.78 (d, 1H, J = 5.5 Hz), 7.31 (d, 1H, J = 8.5 Hz), 7.50 (dd, 1H, J = 8.6, 2.4 Hz), 7.70 (s, 1H), 7.96 (d, 1H, J = 2.5 Hz); MS (EI) m/e 360 (M^+, 0.2), 319 (1.3), 317 (3.9), 262 (4.7), 260 (14.3), 221 (3.1), 219 (9.6). Anal. ($C_{19}H_{25}ClN_4O \cdot {}^1/_2H_2O$) C, H, N.

Methyl 8-Chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepin-3-yl Ketone (9a). A slurry of cuprous iodide (1.42 g, 7.5 mmol, dried by a gentle flame) in 15 mL of dry ethyl ether under argon at 0 °C was treated with methyllithium (1.4 M in hexane, 10.7 mL, 15 mmol). The mixture which resulted was stirred at 0 °C for 15 min to give a clear solution, which was cooled to −78 °C and treated with a solution of acyl chloride **8**[15] (0.7 g, 2.25 mmol) in CH_2Cl_2 (20 mL). The mixture was stirred at −78 °C for 1 h, treated with CH_3OH (1.5 mL), and slowly warmed to room temperature. After addition of saturated aqueous NH_4Cl (3 mL), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with brine (10 mL) and dried ($MgSO_4$). The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography (silica gel, EtOAc) to afford **9a** as white crystals (0.49 g, 76%): mp 226–228 °C; IR (KBr) 3109, 2897, 1719, 1634, 1605, 823 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.61 (s, 3H), 3.20 (s, 3H), 4.35 (br, 1H), 5.35 (br, 1H), 7.41 (d, 1H, J = 8.5 Hz), 7.10 (dd, 1H, J = 8.6, 2.5 Hz), 7.80 (s, 1H), 8.05 (d, 1H, J = 2.4 Hz); MS (EI) m/e 291 (M^+, 28), 289 (M^+, 90), 248 (35), 246 (100), 230 (10), 228 (26). Anal. ($C_{14}H_{12}ClN_3O_2 \cdot {}^3/_4H_2O$) C, H, N.

***n*-Butyl 8-Chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepin-3-yl Ketone (9b)**. Ketone **9b** was prepared in 80% yield from acyl chloride **8** and *n*-butyllithium using the procedure described for ketone **9a**. Purification by flash chromatography (silica gel, EtOAc/hexane (1:1) as eluent) afforded ketone **9b** as white crystals: mp 120–121 °C; IR (KBr) 3122, 2899, 1718, 1629, 1601, 818 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.96 (t, 3H, J = 7.4 Hz), 1.45 (m, 2H), 1.69 (m, 2H), 3.01 (t, 2H, J = 7.2 Hz), 3.21 (s, 3H), 4.29 (br, 1H), 5.30 (br, 1H), 7.35 (d, 1H, J = 8.6 Hz), 7.55 (dd, 1H, J = 8.7, 2.6 Hz), 7.80 (s, 1H), 8.01 (d, 1H, J = 2.4 Hz); MS (EI) m/e 333 (M^+, 34.1), 331 (M^+, 100), 290 (10), 288 (32), 262 (18), 260 (40). Anal. ($C_{17}H_{18}ClN_3O_2$) C, H, N.

2-Amino-5-ethoxybenzoic Acid (13b). A solution of *p*-phenetidine (**10b**) and di-*tert*-butyl dicarbonate (1.05 equiv) was heated to reflux in THF for 2.5 h. After removal of solvent, the residue was recrystallized in ethyl acetate to afford carbamate **11b** as off-white needles in a quantitative yield: mp 118–120 °C; IR (KBr) 3340, 1652, 1611 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.4 (t, 3H, J = 7.0 Hz), 1.5 (s, 9H), 4.0 (q, 2H, J = 7.0 Hz), 6.3 (br, 1H, D_2O exchangeable), 6.85 (d, 2H, J = 8.9 Hz), 7.26 (d, 2H, J = 8.4 Hz). Anal. ($C_{13}H_{19}NO_3$) C, H, N.

A solution of **11b** (10 mmol) in 20 mL of dry THF at −78 °C was treated with *tert*-butyllithium (1.7 M in pentane, 15 mL, 25 mmol) under an atmosphere of nitrogen. The solution was stirred at −78 °C for 20 min and then warmed to −15 °C. After 2 h, the deep red solution was obtained and transferred through a cannula to a slurry of carbon dioxide (excess) in 50 mL of THF at −78 °C. The solution obtained was slowly warmed to room temperature, stirred for 2 h, treated with

saturated aqueous NH_4Cl solution, and acidified with 1 N HCl (pH = 1). The aqueous solution was extracted with ethyl acetate (3 × 50 mL), and the combined extracts were washed with brine and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue which resulted was dissolved in a mixture of THF (10 mL) and concentrated aqueous HCl solution (20 mL). After stirring overnight, the mixture was adjusted to pH = ~5 and extracted with methylene chloride (3 × 50 mL). The combined extracts were washed with brine and dried ($MgSO_4$). After removal of solvent, the residue was purified by flash chromatography (silica gel, ethyl acetate) to afford **13b** (68%) as a yellowish solid. **13b**: mp 179–180 °C; IR (KBr) 2340–3480 (br), 1674 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (t, 3H, J = 7.0 Hz), 3.95 (q, 2H, J = 7.0 Hz), 6.65 (d, 1H, J = 9.0 Hz), 7.0 (dd, 1H, J = 8.9, 2.9 Hz), 7.4 (d, 1H, J = 3.0 Hz). Anal. ($C_9H_{11}NO_3 \cdot {}^1/_8H_2O$) C, H, N.

5-Methoxyisatoic Anhydride (14a) and 5-ethoxyisatoic Anhydride (14b). A solution of **13a** or **13b** (5 mmol) in 20 mL of 1 N HCl was treated with a stream of phosgene for 5 min, and the precipitate which resulted was collected on a filter. The mother liquid was again treated with phosgene to afford the second crop of precipitate. The combined precipitate was washed with cold water and dried in a vacuum oven at 80 °C for 48 h to give **14a** or **14b** as an off-white solid (94% and 95%, respectively). **14a**: mp 340 °C dec; IR (KBr) 3320, 1747 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.8 (s, 3H), 7.1 (d, 1H, J = 8.8 Hz), 7.35 (d, 1H, J = 2.8 Hz), 7.4 (dd, 1H, J = 8.8, 2.9 Hz), 11.6 (br, 1H, D_2O exchangeable); **14a** was used in the next step without further purification and characterization. **14b**: mp 219–221 °C; IR (KBr) 3340, 1754 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.32 (t, 3H, J = 7.0 Hz), 4.05 (q, 2H, J = 6.9 Hz), 7.05 (d, 1H, J = 8.8 Hz), 7.3 (d, 1H, J = 2.9 Hz), 7.4 (dd, 1H, J = 8.7, 2.8 Hz), 11.6 (br, 1H, D_2O exchangeable). Anal. ($C_{10}H_9NO_4$) C, H, N.

7-Methoxy-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5(1*H*)-dione (15a) and 7-ethoxy-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5(1*H*)-dione (15b) were prepared from **14a,b** using the literature procedure.[15] **15a**: mp 211–213 °C; IR (KBr) 3323, 1645 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.3 (s, 3H), 3.8 (s, 3H), 3.9 (s, 2H), 6.9 (d, 1H, J = 8.8 Hz), 7.05 (dd, 1H, J = 8.8, 2.9 Hz), 7.4 (d, 1H, J = 2.9 Hz), 8.2 (br, 1H, D_2O exchangeable). Anal. ($C_{11}H_{12}N_2O_3$) C, H, N. **15b**: mp 185–186.5 °C; IR (KBr) 3312, 1613 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.4 (t, 3H, J = 7.0 Hz), 3.3 (s, 3H), 3.85 (s, 2H), 4.1 (q, 2H, J = 7.0 Hz), 6.9 (d, 1H, J = 8.7 Hz), 7.02 (dd, 1H, J = 8.7, 2.9 Hz), 7.4 (d, 1H, J = 2.7 Hz), 7.9 (br, 1H, D_2O exchangeable). Anal. ($C_{12}H_{14}N_2O_3$) C, H, N.

Ethyl 8-methoxy-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (16a) and ethyl 8-ethoxy-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo-[1,5-*a*][1,4]benzodiazepine-3-carboxylate (16b) were prepared from **15a,b** using the procedure described in the literature.[15] **16a**: mp 232–234 °C; IR (KBr) 1723 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (t, 3H, J = 7.1 Hz), 3.25 (s, 3H), 3.9 (s, 3H), 4.4 (br, 1H), 4.45 (q, 2H, J = 7.2 Hz), 5.2 (br, 1H), 7.15 (dd, 1H, J = 8.8, 2.9 Hz), 7.32 (d, 1H, J = 8.8 Hz), 7.55 (d, 1H, J = 2.9 Hz), 7.82 (s, 1H); MS (EI) m/e 315 (M^+, 32.2), 269 (37.3), 241 (100), 213 (17.3). Anal. ($C_{16}H_{17}N_3O_4$) C, H, N. **16b**: mp 151–152 °C; IR (KBr) 1721 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (dt, 6H, J = 7.0, 1.9 Hz), 3.23 (s, 3H), 4.15 (m, 2H), 4.35 (br, 1H), 4.45 (q, 2H, J = 6.9 Hz), 5.2 (br, 1H), 7.13 (dd, 1H, J = 8.7, 2.9 Hz), 7.33 (d, 1H, J = 8.8 Hz), 7.53 (d, 1H, J = 2.9 Hz), 7.85 (s, 1H); MS (EI) m/e 329 (M^+, 29.0), 283 (31.6), 255 (100), 227 (15.3). Anal. ($C_{17}H_{19}N_3O_4 \cdot {}^1/_4H_2O$) C, H, N.

7-Methoxy-2-(4-methoxyphenyl)pyrazolo[4,3-*c*]quinolin-3-one (18a) and 8-chloro-2-(4-methoxyphenyl)pyrazolo[4,3-*c*]quinolin-3-one (18b) were prepared from **17a**,[35] **17b**,[35] and (4-methoxyphenyl)hydrazine using the procedure described in the literautre.[18] **18a**: mp 250–252 °C; IR (KBr) 3445, 1637 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.75 (s, 3H), 3.85 (s, 3H), 7.0 (d, 2H, J = 9.1 Hz), 7.15 (dd, 2H, J = 6.3, 2.9 Hz), 8.1 (m, 3H), 8.65 (s, 1H), 12.6 (br, 1H, D_2O exchangeable); MS (EI) m/e 321 (M^+, 100), 306 (50.2), 160 (18.5), 158 (30.7). Anal. ($C_{18}H_{15}N_3O_3 \cdot {}^1/_2H_2O$) C, H, N. **18b**: mp 326–328 °C; IR (KBr) 3434, 1623 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.78 (s, 3H), 7.0 (d, 2H, J = 9.0 Hz), 7.7 (s, 2H), 8.12 (m, 3H), 8.7 (s, 1H), 12.85 (br, 1H, D_2O exchangeable); MS (EI) m/e 327 (M^+, 32.4), 325 (M^+, 100), 310 (64.6), 162 (42.0), 135 (29.7). Anal. ($C_{17}H_{12}ClN_3O_2$) C, H, N.

Supplementary Material Available: The coordinates for represented compounds (both *syn* and *anti* conformations of **5b**, **9b**, and **24**, as well as **28** and diazepam) (24 pages). Ordering information given on any current masthead page.

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