Potential Anxiolytic Agents. Pyrido[1,2-*a*]benzimidazoles: A New Structural Class of Ligands for the Benzodiazepine Binding Site on GABA-A Receptors

Bruce E. Maryanoff,* Winston Ho,†
David F. McComsey, Allen B. Reitz, Philip P. Grous,†
Samuel O. Nortey, Richard P. Shank,
Barry Dubinsky, Russell J. Taylor, Jr., and
Joseph F. Gardocki

Drug Discovery, The R. W. Johnson Pharmaceutical Research Institute, Spring House, Pennsylvania 19477

Received October 6, 1994

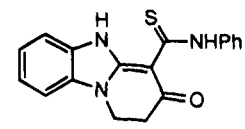
Receptors for γ-aminobutyric acid of the "A" variety (GABA-A receptors), the most abundant inhibitory receptors in mammalian brain, are structurally constituted as macromolecular heteropentameric assemblies (combinations of α, β, and γ/δ protein subunits).[1] Each GABA-A receptor complex comprises a chloride ion channel that not only controls chloride flux across neuronal membranes, but also bears multiple recognition sites for small modulatory molecules, such as benzodiazepines, barbiturates, picrotoxin, and certain steroids.[1] When GABA interacts with its receptor, the ion channel is opened, chloride influx is enhanced, the membrane is hyperpolarized, and the cell becomes less responsive to excitatory stimuli. This GABA-induced ion current can be regulated by diverse agents,[2] particularly those which bind to the so-called "benzodiazepine (BZD) receptor." Two opposing effects are mediated, one that amplifies ("positive" modulation) and one that attenuates ("negative" modulation) the action of GABA,[2] which define agonists and inverse agonists, respectively. Antagonists at the BZD binding site block the effects of both agonists and inverse agonists by competitive inhibition. Hence, a series of compounds can contain ligands that bind equally well to the BZD site but have opposite modulatory effects, or no effects at all. By the same token, a series can display a continuum of activity that translates into a wide spectrum of significant central nervous system (CNS) pharmacology.[3,4] Indeed, agonists generally produce muscle relaxant, hypnotic, sedative, anxiolytic, and/or anticonvulsant effects, while inverse agonists produce proconvulsant, anti-inebriant, and anxiogenic effects.[4]

The benzodiazepines, β-carbolines, imidazopyridines, pyrazoloquinolines, and imidazoquinoxalines (Scheme 1) represent major families of compounds with high affinity for the BZD binding site, and with anxiolytic properties.[4e,5-7] Most of the drugs in clinical use, such as diazepam, are "full" agonist-type ligands at the BZD site and exhibit anticonvulsant, sedative, and muscle relaxant effects in addition to anxiolytic properties. By contrast, partial agonists are considered to be likely to afford anxiolytic activity without the undesirable side effects.[4d,f,6] Within this context, we report a new structural class of high-affinity ligands for the BZD binding site—the pyrido[1,2-*a*]benzimidazoles (PBI's)—represented by prototype **1a** (RWJ-16979; Scheme 1). Compounds in the PBI series not only span the gamut of GABA receptor-based biological activity, from agonists to antagonists, but also furnish partial agonists with potential as novel anxiolytic agents.

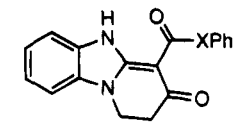
Synthetic Chemistry. The tricyclic nucleus for the PBI series was synthesized by the route shown in Scheme 2. 2-Nitroaniline was reacted with acrylonitrile in dioxane, and the resultant propionitrile was hydrogenated to a phenylenediamine (87%). The benzimidazole nucleus was formed by using carbethoxyacetimidate hydrochloride (refluxing ethanol; 81%),[8] the nitrile group was converted to an ester group, and Dieckmann condensation furnished keto ester **2** (83%).[9] Key intermediate **2** was reacted with various amines, especially anilines, usually in refluxing xylenes, to obtain the carboxamide targets **1** (Table 1) in high yield. Phenyl derivative **1a** serves as a prototype of the PBI series.

The N-alkylated B-ring analogues were prepared as shown in Scheme 3. Benzimidazole **2** was reacted with an alkyl halide in the presence of sodium hydride to afford the *N*-alkyl ester (70–80%), which readily decarboxylated to enone **4** on conversion to the acid (ca. 100%). Reaction of the enone with isocyanates gave the *N*-alkyl targets **3** (68–76%). Direct alkylation of **1**, as depicted in Scheme 3, gave modest to poor results from one case to another.

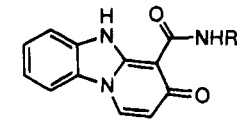
Compound **5** was prepared from **1a** with Lawesson's reagent (toluene, 95 °C; 74%).[10] *N*-Methylamide **6** was synthesized from **2** and *N*-methylaniline (refluxing xylenes; 82%). Similarly, reaction of **2** with phenol or thiophenol gave **7** (38%) and **8** (16%), respectively. Oxidation of **1a** with MnO_2[11] at 23 °C or with Pd/C[12] in refluxing xylenes gave **9** (ca. 50%); however, the oxidation of **1e** to **10** required DDQ[13] in refluxing dioxane (22%).



5



6 X = NMe
7 X = O
8 X = S



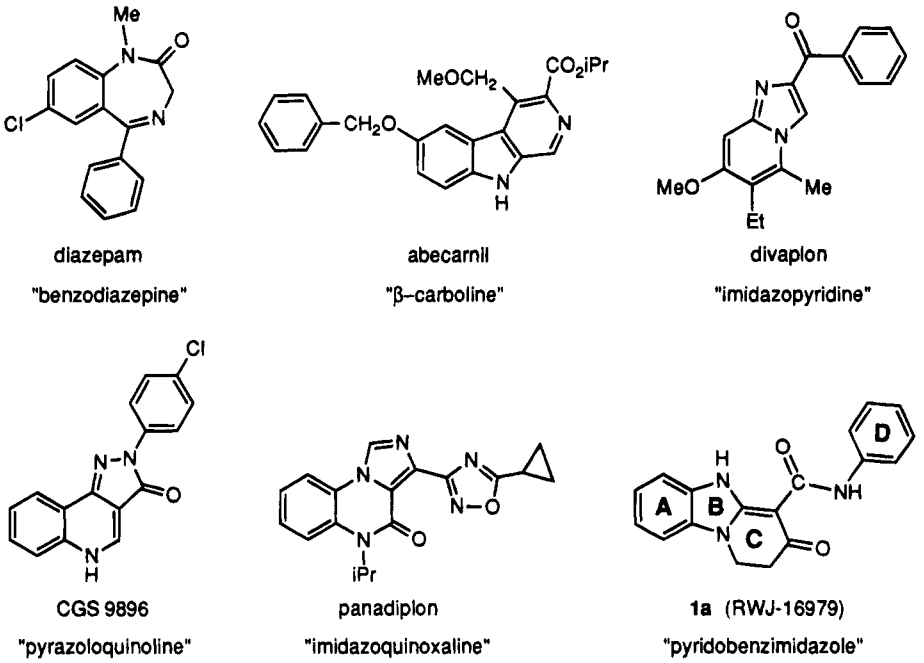
9 R = Ph
10 R = 2-FPh

Structural Aspects. Compounds with a B-ring hydrogen (as in **1a**) can exist in at least two possible tautomers, e.g., keto (**1a**) and enol (**1a'**).[14] Carbon-13 NMR data indicate that the keto form greatly predominates in solution.[14] An X-ray structure determination for **1a** also shows the keto form, with a substantially planar tricyclic portion (monoclinic space group $P2_1/N$; $Z = 4$; 2221 observed out of 2847 measured reflections; $R = 0.038$).[15] The carboxamide is coplanar with the π system of the tricycle and is hydrogen bonded to the adjacent polar atoms on both sides (NH proximate to the C-ring carbonyl oxygen). The saturated segment of the C ring is slightly puckered out of the plane defined by the tricyclic array. An X-ray determination on **3a**

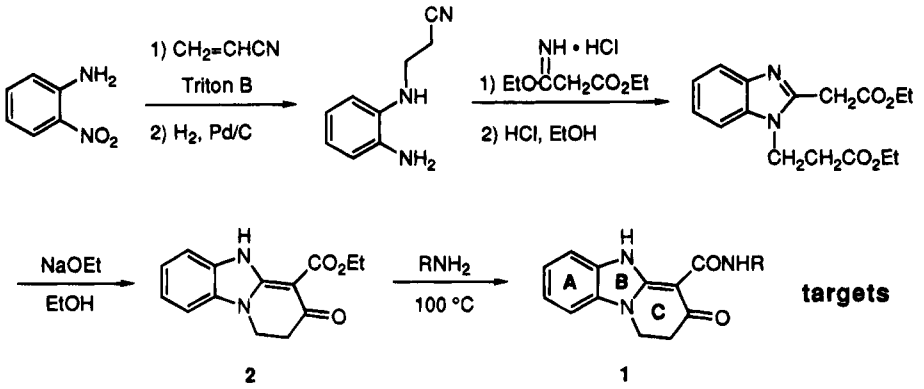
* Address correspondence to this author at the R. W. Johnson Pharmaceutical Research Institute.
† Janssen Research Foundation, Spring House, PA.

0022-2623/95/1838-0016$09.00/0

Scheme 1. Comparison of Structural Series



Scheme 2. Synthesis of the Parent PBI System



revealed a similar overall structure for the tricycle (orthorhombic space group *Pbca*; $Z = 8$; 2079 observed out of 2847 measured reflections; $R = 0.090$); however, the amide is rotated ca. 30° out of the plane of the tricycle, with a hydrogen bond between the NH and the C-ring carbonyl.[15]

Biological Testing. The PBI compounds were examined for activity in vitro and in vivo (Table 1). Affinity (IC_{50} value) to the BZD site of the GABA-A receptor was determined by competition with the radioligand [^{3}H]flunitrazepam.[16] Compounds were tested at five concentrations in a tissue preparation from rat cerebral cortex. Binding was measured in the absence and presence of GABA (1 mM) to obtain the GABA shift (GS) value, from which one can assess agonist, antagonist, and inverse agonist activity for a ligand (Figure 1).[4] This in vitro classification method is useful for estimating the functional properties of test compounds. Thus, a "full agonist" would have a GS greater than or equal to 2.0, an antagonist would have a GS in the vicinity of 1.0, an inverse agonist would have a GS of less than or equal to 0.7, and a partial agonist would have a GS in the range of 1.0–1.5.[4g]

Test compounds were assessed for in vivo biological activity in mice and rats by using standard assays. Following intraperitoneal (ip) administration, we measured the ability of compounds to block the tonic-clonic component of seizures induced by pentylenetetrazole (metrazol, PTZ) in mice (ED_{50} value)[17] and to release (disinhibit) behavior that had been suppressed by punishment in rats ("conflict" test).[18] This "conflict" test affords anxiolytic activity as a minimum effective dose (MED).

Prototype **1a** has strong affinity ($IC_{50} = 9.1$ nM) for the BZD site and a GABA shift of 1.5; it is also moderately potent in vivo in the anticonvulsant and anxiolytic assays. The cyclohexyl analogue **1k** shows a 15-fold reduction in affinity and moderate in vivo potency. The cyclobutyl (**1l**; RWJ-46891) and cyclopropyl (**1m**; RWJ-45683) derivatives are interesting in that affinity for the BZD site is substantially restored; however, the GABA shifts are now in the range of antagonists (0.8–1.2). The attenuation of in vivo activity for **1l**, and particularly for **1m**, also suggests antagonism. In fact, on studying **1m** for its ability to antagonize chlordiazepoxide-induced impairment of horizontal screen performance in mice,[19] we found 100% antagonism at 40 mg/kg ip ($p < 0.05$). For reference purposes, at 40 mg/kg the known antagonists CGS-8216 (GS = 1.1; data not shown) and Ro-15-4513 (GS = 0.72) exhibit 100% and 67% antagonism, respectively. Consequently, we came to recognize that PBI compounds

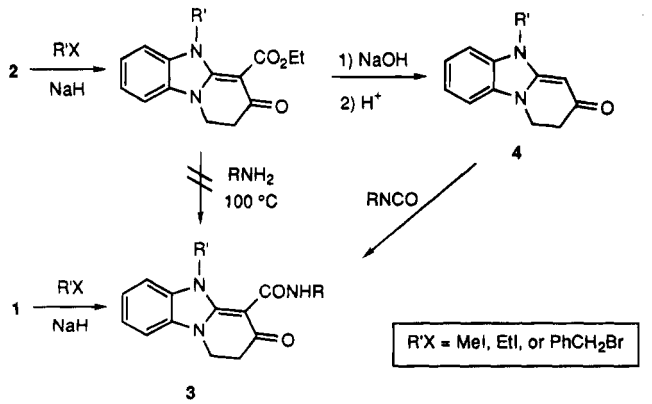
Table 1. Chemical Properties and Biological Data for Selected PBI Derivatives[a]

compd	R	mp, °C (solv)[b]	IC_{50} (nM) no GABA[c]	IC_{50} (nM) GABA[c]	GS[d]	metrazol (ip) ED_{50} (mg/kg)[e]	conflict (ip) MED (mg/kg)
			Section A				
1a	Ph	224–225 (C/E)	9.1	5.9	1.5	5.2 (2.2–13.3)	10
1b	4-ClPh	258–262 (D)	620	265	2.3	8.9 (3.1–30)	1
1c	3-ClPh	260–262 (C/T)	120	95	1.3	>30	>10
1d	2-ClPh	228–230 (D)	12	5.3	2.3	0.95 (0.39–2.6)	10
1e	2-FPh	247–249 (THF)	1.7	1.4	1.2	0.16 (0.080–0.29)	1
1f	4-MeOPh	196–197 (D/E)	41	16	2.6	2.0 (0.092–3.8)	10
1g	3-MeOPh	248–250 (C/E)	26	16	1.6	>10	5
1h	2-MeOPh	229–230 (D/E)	990	940	1.1	>10	>10
1i	2,6-Cl_2Ph	134–141 (D/E)	320	170	1.9	>30	>10
1j	2,6-F_2Ph	216–218 (D/E)	2.8	1.7	1.6	0.85 (0.37–2.6)	0.1
1k	*c*-C_6H_{11}	158–160 (C/E)	140	72	2.0	3.0 (1.3–5.8)	10
1l	*c*-C_4H_7	217–219 (AE)	30	25	1.2	>30	10
1m	*c*-C_3H_5	215–217 (D/X)	16	18	0.87	>10	>10
1n	H	241–243 (D/EA)	2400	6000	0.40	>30	10
2		233–235 (C/M)	675	840	0.68	>10	
5		224–227 (D/E)	360	320	1.1	>10	>10
6		195–197 (C/EE)	>10000	>10000		>10	>10
7		176–178 (D/E)	200	150	1.4	9.9 (4.4–71)	>10
8		220–222 (D/A)	29	26	1.1	>30	>10
9		264–266 (C/EA)	24	14	1.7	0.39 (0.12–0.81)	10
10		237–238 (D/M)	0.23	0.11	2.1	0.088 (0.023–0.23)	0.1
diazepam			28	13	2.2	0.11 (0.058–0.15)	5
Ro-15-1788			6.2	5.6	1.1	>10	>10
Ro-15-4513			4.9	6.8	0.72	>10	>10

compd	R′	R	mp, °C (solv)[b]	IC_{50} (nM) no GABA[c]	IC_{50} (nM) GABA[c]	GS[d]	metrazol (ip) ED_{50} (mg/kg)[e]	conflict (ip) MED (mg/kg)
				Section B				
3a	Me	Ph	205–206 (D/E)	5.8	2.1	2.8	0.13 (0.038–0.28)	1
3b	Me	2-FPh	196–197 (AE)	0.42	0.26	1.6	0.019 (0.0064–0.033)	0.03
3c	Et	Ph	223–224 (D/E)	2.1	1.4	1.6	0.083 (0.047–0.13)	0.3
3d	$PhCH_2$	Ph	216–218 (D/E)	12	2.7	4.6	1.4 (0.83–2.2)	10

[a] Structures for **1** and **2** are in Scheme 2. Under the heading "R", "Ph" represents a benzene ring with an appropriate number of hydrogen atoms to satisfy the substitution. All compounds were isolated and purified in unadducted form (i.e., no acid or base addition salts); thus, they are represented by standard molecular formulas except for the following solvates: **1e** (0.25 molar equiv of THF), **1h** (0.6 C_2H_5OH), **1j** (0.05 CH_2Cl_2), **1o** (0.25 H_2O), **7** (0.25 CH_2Cl_2), **10** (0.25 H_2O). Microanalytical data (C, H, N; water where necessary) were within the accepted range. Information on biological testing is provided in the text. [b] Mp values are corrected to a set of standards. The recrystallization solvent is given in parentheses: A = acetone, AE = 95% EtOH, C = $CHCl_3$, D = CH_2Cl_2, E = EtOH, EA = ethyl acetate, EE = ethyl ether, M = MeOH, T = toluene, THF = tetrahydrofuran, X = xylenes. [c] The 95% confidence limits are contained in supplementary material (see paragraph at the end of this paper). [d] GS = GABA shift = IC_{50}(no GABA)/IC_{50}(GABA). [e] For ED_{50} values the 95% confidence interval are given in parentheses.

Scheme 3. Synthesis of PBI Analogues with an Alkylated B Ring



with high affinity for the BZD site (IC_{50} < 100 nM), low GABA shifts (0.8–1.3), and little or no activity in the metrazol or conflict assays are likely to be antagonists.

A small sampling of results from our extensive study of aromatic substitution[20] of the D ring of **1a** is offered in this preliminary report. Although the affinity of 4-chloro analogue **1b** is attenuated (IC_{50} = 620 nM), there is still significant in vivo activity. The 2-chloro analogue (**1d**) has properties similar to **1a**, but the 3-chloro analogue (**1c**) shows moderate affinity (IC_{50} = 120 nM), a low GABA shift (1.3), and virtually no in vivo activity. It is conceivable that antagonist properties are dominant with **1c**. The methoxy series shows a different profile. The 4-methoxy analogue (**1f**) has a nearly 5-fold decrease in affinity relative to **1a** and a similar potency in vivo; the 3-methoxy analogue (**1g**) has slightly more affinity than **1f**, but modest-to-mixed in vivo potency; and the 2-methoxy analogue (**1h**), unlike **1d**, has low affinity (IC_{50} = 990 nM) and no in vivo activity.[21] Certain fluorine substitution is especially noteworthy. The 2-fluoro analogue (**1e**) has excellent affinity (IC_{50} = 1.7 nM) and very good potency in both in vivo assays; the GABA shift for **1e** suggests a partial agonist. Additionally, 2,6-difluoro derivative **1j** exhibits similar favorable characteristics, including remarkable potency in the important "conflict" test (MED = 0.1 mg/kg). By contrast, the 2,6-dichloro compound (**1i**) is not interesting at all. Also, substituents at the 4-position that are moderately larger than F but strongly electron-withdrawing (e.g., 4-CN and 4-NO_2) show substantially reduced affinity and in vivo potency compared with **1a**.[20]

N-Methyl derivative **3a** has a similar affinity to **1a**, but a much higher, full agonist GABA shift (2.8) and a 10–30-fold enhancement of in vivo potency. Introduction of a 2-fluoro substituent into **3a**, in the form of **3b**

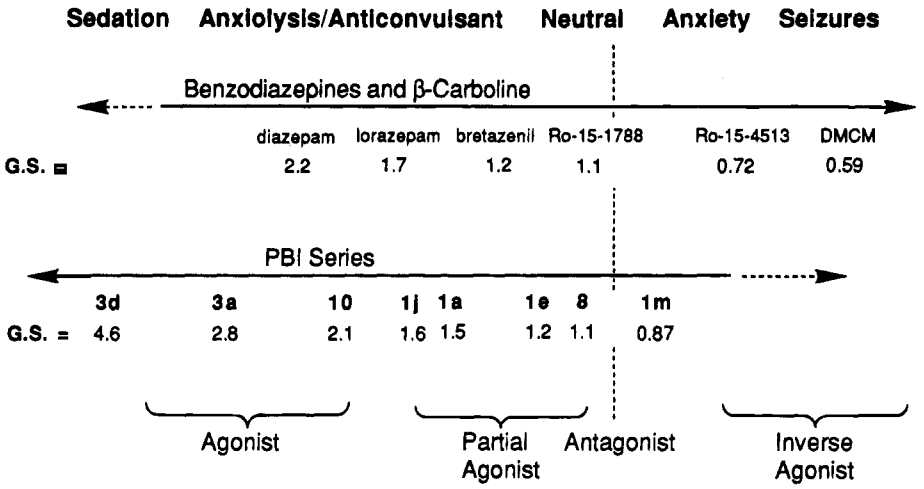


Figure 1. Spectrum of activity for the BZD and PBI series as deduced from GABA-shift data for representative compounds. DMCM is a β-carboline inverse agonist.

(RWJ-46771), proved even more exceptional by achieving subnanomolar affinity (IC_{50} = 0.42 nM) and potencies of 0.019 mg/kg (PTZ) and 0.03 mg/kg (conflict) in the in vivo assays! Moreover, since **3b** possesses a GABA shift in the range of a partial agonist, it offers promise as a potential anxiolytic agent devoid of unwanted side effects. The benzyl analogue, **3d**, has a good affinity for the BZD site (IC_{50} = 12 nM) but, more importantly, a very high GABA shift of 4.6. This result suggests that **3d** is a pure agonist, which seems to be borne out by its performance in vivo.

Ethyl ester **2** and carboxamide **1n** are weak ligands for the BZD site and show uninteresting in vivo activity. Although phenyl ester **7** shows just a modest increase in activity over **2**, phenylthio ester **8** shows significant affinity (IC_{50} = 29 nM). However, **8** lacks in vivo activity, which may be attributed to antagonist behavior, reflected in the GABA shift of 1.1. Surprisingly, tertiary carboxanilide **6** is devoid of binding affinity, possibly arising from steric bulk or conformational restriction of the amide group. Unsaturated C-ring derivative **9** has slightly less affinity but shows worthwhile in vivo activity. Significantly, the corresponding 2-fluoro analogue, **10** (RWJ-45788), has subnanomolar affinity (IC_{50} = 0.23 nM) and exceptional in vivo potency (0.088 mg/kg in PTZ; 0.1 mg/kg in conflict).

Conclusion. We have discovered a new class of GABA-A receptor modulators, the pyrido[1,2-*a*]benzimidazoles (PBI's), which act via the BZD binding site. A range of activity from full agonism to antagonism was observed, the extrema being represented by **3d** and **1m**, respectively (Figure 1). This PBI series contains compounds with subnanomolar affinity at the BZD site (viz. **3b**; **10**) and with very potent anxiolytic activity (μg/kg MED in the conflict test) on i.p. administration (viz. **1j**, **3b**, **3c**, **10**). On the basis of anticonflict potency and GABA shifts indicative of partial agonism (GS = 1.0–1.5), **1e** and **1j** have promise as anxiolytic agents with limited side effects.[22] These preliminary results establish a firm platform to evolve drug candidates for the treatment of anxiety.

Acknowledgment. We are grateful to Prof. Roy Olofson (Pennsylvania State University) for the X-ray diffraction results. We thank Dr. Ellen Baxter for helpful discussion, and William Baldy, Alexandre Carter, and Craig Schneider for technical assistance.

Supplementary Material Available: Table of IC_{50} values for the binding assay with the Hill slopes and confidence limits (2 pages). Ordering information is given on any current masthead page.

References

(1) (a) DeLorey, T. M.; Olsen, R. W. γ-Aminobutyric Acid$_A$ Receptor Structure and Function. *J. Biol. Chem.* **1992**, *267*, 16747–16750. (b) Macdonald, R. L.; Olsen, R. W. GABA(A) Receptor Channels. *Annu. Rev. Neurosci.* **1994**, *17*, 569–602. (c) Wisden, W.; Laurie, D. J.; Monyer, H.; Seeburg, P. H. The Distribution of 13 GABA$_A$ Receptor Subunit mRNAs in the Rat Brain. I. Telencephalon, Diencephalon, Mesencephalon. *J. Neurosci.* **1992**, *12*, 1040–1062. (d) Laurie, D. J.; Seeburg, P. H.; Wisden, W. The Distribution of 13 GABA$_A$ Receptor Subunit mRNAs in the Rat Brain. II. Olefactory Bulb and Cerebellum. *J. Neurosci.* **1992**, *12*, 1063–1076. (e) Olsen, R. W.; Tobin, A. J. Molecular Biology of GABA$_A$ Receptors. *FASEB J.* **1990**, *4*, 1469–1480. (f) Sieghart, W. Multiplicity of GABA$_A$-Benzodiazepine Receptors. *Trends Pharmacol. Sci.* **1989**, *10*, 407–411.

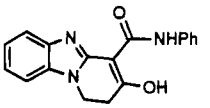
(2) Puia, G.; Vicini, S.; Seeburg, P. H.; Costa, E. Influence of Recombinant γ-Aminobutyric Acid$_A$ Receptor Subunit Composition on the Action of Allosteric Modulators of γ-Aminobutyric Acid-Gated Cl^- Currents. *Mol. Pharmacol.* **1991**, *39*, 691–696.

(3) Takada, S.; Shindo, H.; Sasatani, T.; Chomei, N.; Matsushita, A.; Eigyo, M.; Kawasaki, K.; Murata, S.; Takahara, Y.; Shintaku, H. Thienylpyrazoloquinolines: Potent Agonists and Inverse Agonists to Benzodiazepine Receptors. *J. Med. Chem.* **1988**, *31*, 1738–1745.

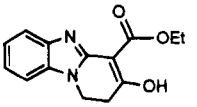
(4) For other background information, see: (a) Mohler, H. GABAergic Synaptic Transmission. Regulation by Drugs. *Arzneim.-Forsch./Drug Res.* **1992**, *42*, 211–214. (b) Haefely, W.; Kyburz, E.; Gerecke, M.; Mohler, H. Recent Advances in the Molecular Pharmacology of Benzodiazepine Receptors and in the Structure-Activity Relationships of Their Agonists and Antagonists. *Adv. Drug Res.* **1985**, *14*, 165–322. (c) Skolnick, P.; Paul, S. M. *GABA and Benzodiazepine Receptors*; Squires, R. F., Ed.; CRC Press: Boca Raton, 1987; pp 99–102. (d) Haefely, W.; Martin, J. R.; Schoch, P. Novel Anxiolytics That Act as Partial Agonists at Benzodiazepine Receptors. *Trends Pharmacol. Sci.* **1990**, *11*, 452–456. (e) Gammill, R. G.; Carter, D. B. Neuronal BZD Receptors: New Ligands, Clones, and Pharmacology. *Annu. Rep. Med. Chem.* **1993**, *28*, 19–27. (f) Haefely, W.; Facklam, M.; Schoch, P.; Martin, J. R.; Bonetti, E. P.; Moreau, J.-L.; Jenck, F.; Richards, J. G. Partial Agonists of Benzodiazepine Receptors for the Treatment of Epilepsy, Sleep, and Anxiety Disorders. *GABAergic Synaptic Transmission*, Biggio, G., Concas, A., Costa, E., Eds.; Raven Press: New York, 1992; pp 379–394. (g) Williams, M.; Olsen, R. W. Benzodiazepine Receptors and Tissue Function. *Receptor Pharmacology and Function*, Williams, M., Glennon, R. A., Timmermans, P. B. M. W. M., Eds.; Marcel Dekker: New York, 1989, pp 385–413.

(5) (a) Gardner, C. R.; Tully, W. R.; Hedgecock, C. J. R. The Rapidly Expanding Range of Neuronal Benzodiazepine Receptor Ligands. *Prog. Neurobiol.* **1993**, *40*, 1–61. (b) Kerr, D. I. B.; Ong, J. GABA Agonists and Antagonists. *Med. Res. Rev.* **1992**, *12*, 593–636.

(6) TenBrink, R. E.; Im, W. B.; Sethy, V. H.; Tang, A. H.; Carter, D. B. Antagonist, Partial Agonist, and Full Agonist Imidazo-[1,5-*a*]quinoxaline Amides and Carbamates Acting through the $GABA_A$/Benzodiazepine Receptor. *J. Med. Chem.* **1994**, *37*, 758–768.
(7) Stephens, D. N.; Schneider, H. H.; Kehr, W.; Andrews, J. S.; Rettig, K.-J.; Turski, L.; Schmiechen, R.; Turner, J. D.; Jensen, L. H.; Petersen, E. N.; Honore, T.; Hansen, J. B. Abecarnil, a Metabolically Stable, Anxioselective β-Carboline Acting at Benzodiazepine Receptors. *J. Pharmacol. Exp. Ther.* **1990**, *253*, 334–343.
(8) De Selms, R. C. Benzimidazoles. II. Synthesis of N-Heterocyclic Ring Systems Containing 1,2-Fused Benzimidazole Moieties. *J. Org. Chem.* **1962**, *27*, 2165–2167.
(9) Ohta, S.; Yuasa, T.; Narita, Y.; Kawasaki, I.; Minamii, E.; Yamashita, M. Synthesis and Application of Imidazole Derivatives. Synthesis of Pyrido[1,2–*a*]Benzimidazolone Derivatives. *Heterocycles* **1991**, *32*, 1923–1931.
(10) Raucher, S.; Klein, P. A. Convenient Method for the Selective Reduction of Amides to Amines. *Tetrahedron Lett.* **1980**, *21*, 4061–4064.
(11) Fatiadi, A. J. Active Manganese Dioxide Oxidations in Organic Chemistry Part II. *Synthesis* **1976**, 133–167.
(12) Mosettig, E.; Duvall, H. Studies in the Phenanthrene Series. XIV. The Preparation of 1- and 4- Phenanthrol. *J. Am. Chem. Soc.* **1937**, *59*, 367–369.
(13) Burn, D.; Petrow, V. Modified Steroid Hormones. Part XXVIII. 17α-Acetoxy-4α-methyl-5α-pregn-1-ene-3,20-dione. *J. Chem. Soc.* **1962**, 1223–1126.
(14) ^{13}C NMR data ($CDCl_3$ or DMSO-d_6) for **1a** and **2** indicate a strong population of the keto tautomer (unpublished results). In the literature, enol structure **2'** was incorrectly assigned to **2**.[9]



1a'



2'

(15) Details relating to the X-ray crystallography are available on request. This work, along with other structural studies,[14] will be published separately.
(16) Wood, P. L.; Loo, P.; Braunwalden, A.; Yokoyama, N.; Cheney, D. L. In Vitro Characterization of Bendodiazepine Receptors Agonists, Antagonists, Inverse Agonists and Agonist/Antagonists *J. Pharmacol. Exp. Ther.* **1984**, *231*, 572–576.
(17) Swinyard, E. A. Laboratory Assay of Clinically Effective Anti-epileptic Drugs. *J. Am. Pharm. Assoc.* **1949**, *38*, 201–204.
(18) Vogel, J. R.; Beer, B.; Clody, D. E. A Simple and Reliable Conflict Procedure for Testing Anti-Anxiety Agents. *Psychopharmacology* **1971**, *21*, 1–7.
(19) Coughenour, L. L.; McLean, J. R.; Parker, R. B. A New Device for the Rapid Measurement of Impaired Motor Function in Mice. *Pharmacol. Biochem. Behav.* **1977**, *6*, 351–353.
(20) Maryanoff, B. E.; McComsey, D. F.; Ho, W. World Patent appl. WO9404532-A1, 3 Mar 1994.
(21) Although the GABA shift for **1h** is low (1.1), we tend to assign less meaning to GABA shift values for compounds with relatively weak affinity for the BZD site (IC_{50} > 500 nM).
(22) Potokar, J.; Nutt, D. J. Anxiolytic Potential of Benzodiazepine Receptor Partial Agonists. *CNS Drugs* **1994**, *1*, 305–315.

JM9406514