and Cross.[23] Male crossbreed guinea pigs weighing 350–500 g, 18-h fasted, were used. Under sodium pentobarbital narcosis, blood was taken from the abdominal aorta and was rendered nonclotting by adding a 3.8% (w/v) sodium citrate solution (final volume ratio 1:10). A plasma rich in platelets was then obtained as supernatant by centrifuging. Aggregation was triggered by adding (a) ADP at doses ranging from 0.25 to 2 μM/mL, (b) collagen (from equine tendon) at doses ranging from 0.8 to 2.4 μg/mL, and (c) thrombin at doses ranging from 0.312 to 1.25 units/mL. Incubation of the platelets with the test compounds was carried out for 10 min at room temperature at a dose equimolar to the minimal dose of ASA (66 μM dissolved in 0.3 M CH_3COONa) which completely inhibits platelet aggregation. The inhibiting action was expressed as percent of inhibition by comparing the aggregation curve of the test compound with that of the control.

Antithrombotic Activity in Vivo. The determination was carried out by a modification of the method of Minno and Silver.[24] Male Swiss mice weighing 20–30 g were divided in three groups of 10. Groups 1 and 2 were treated with test compounds and the reference drug (ASA), respectively, both dissolved in 1% (hydroxymethyl)cellulose and orally administered in a volume of 50 mL/kg. The dose of the test compound was equimolar to that of ASA (20 mg/kg). One hour after medication, groups 1 and 2, along with group 3 (controls), received a thrombotic mixture [fetal bovine collagen (200 μg/mL) and adrenaline (200 μM)] in a volume of 10 mL/kg administered iv in the tail (before injection animals were warmed at 27 °C for 30 min). Death of the animals or paralysis for more than 15 min of the hind limbs were considered as thrombotic effects. The antithrombotic activity was characterized as percent protection (%P) by relating the number of the thrombotic effects in group 1 (treated) to those of group 3 (controls), according to the following formula: $\%P = (N_c - N_t)/N_c \times 100$. The protection of the test compound was then compared to that of the reference drug (group 2).

Registry No. (±)-**3a**, 121866-02-2; (±)-**3b**, 103603-13-0; (±)-**3c**, 121866-03-3; (+)-**3c**, 103602-97-7; (−)-**3c**, 103602-95-5; **3d**, 121866-04-4; (±)-**3e**, 110138-94-8; (±)-**3f**, 110138-93-7; (±)-**3g**, 121866-05-5; (±)-**4b**, 103603-08-3; (±)-**4c**, 103602-79-5; **5c**, 88611-67-0; **5e**, 102873-24-5; **5f**, 58161-21-0; **6c**, 103602-85-3; **6e**, 110139-16-7; **6f**, 110139-13-4; (±)-**7c**, 103602-78-4; (+)-**7c**, 103602-89-7; (+)-**7c** (+)-α-methylbenzylamine salt, 103602-90-0; (−)-**7c**, 103602-91-1; (±)-**7e**, 110139-07-6; (±)-**7f**, 110139-08-7; **9c**, 103602-86-4; (±)-**10c**, 121866-01-1; 11, 104120-90-3; **12**, 25823-49-8; glyoxylic acid, 298-12-4; (+)-α-methylbenzylamine, 3886-69-9; (−)-α-methylbenzylamine, 2627-86-3; 2-chloropropionyl chloride, 7623-09-8.

(23) Born, G. V. R.; Cross, M. J. *J. Physiol.* **1963**, *168*, 178.
(24) Minno, G.; Silver, N. J. *J. Pharm. Exp. Ther.* **1983**, *225*, 57.

Novel Benzodiazepine Receptor Partial Agonists: Oxadiazolylimidazobenzodiazepines

Frank Watjen,[†] Raymond Baker,[‡] Mogens Engelstoff,[†] Richard Herbert,[‡] Angus MacLeod,[‡] Anthony Knight,[§] Kevin Merchant,[‡] Jonathan Moseley,[‡] John Saunders,*[,‡] Christopher J. Swain,[‡] Erik Wong,[§] and James P. Springer[∥]

Ferrosan Research Division, Sydmarken 5, 2860 Soeborg, Denmark, Chemistry and Biochemistry Departments, Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR, United Kingdom, and Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey 07065. *Received August 18, 1988*

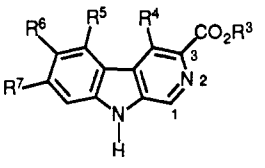
The synthesis and biochemical evaluation of a series of oxadiazole derivatives of imidazobenzodiazepines related to the benzodiazepine antagonist Ro 15-1788 (**2a**) are reported. Although the oxadiazole ring is seen as an isosteric replacement for the ester linkage, significant differences in structure–activity trends were observed. Specifically, oxadiazoles **9**–**12** invariably had increased receptor efficacy (as witnessed by measurements of the GABA shift) relative to the corresponding ester. Additionally, and in direct contrast to the classical agonists such as diazepam, affinity for the benzodiazepine receptor was enhanced by a 7- rather than 8-halo substituent. The results are discussed in terms of a six-point receptor-binding model originally based on the X-ray structure of **2a**. For comparison, the crystal structures of two representative oxadiazole derivatives, **10h** and **12o**, having a 6-oxo and 6-phenyl group, respectively, were determined and the data incorporated into a modified binding model to account for the greater efficacy of these compounds. It is concluded that the antagonist behavior of **2a** relies upon the hydrogen-bond-acceptor properties of the ester carbonyl oxygen whereas for the oxadiazole series this site is localized at the imidazole nitrogen.

It is well-established that benzodiazepines and related ligands interact with a specific site ("the benzodiazepine receptor") that is closely associated with a neuro-inhibitory, postsynaptic $GABA_A$ receptor and a chloride ionophore channel.[1,2] The efficiency of coupling of the $GABA_A$ receptor to the chloride ion effector mechanism can be modified by several series of compounds that bind at this site. Uniquely, it has been shown that this receptor can be occupied by ligands having a continuum of intrinsic efficacy, from positive efficacy (anxiolytic, anticonvulsant, and sedative agents), through nil intrinsic efficacy (receptor antagonists) to negative intrinsic efficacy (proconvulsant, anxiogenic agents).[3,4] The existence of these three categories would also imply that partial agonists and partial inverse agonists exist. Partial inverse agonists may be useful as cognition enhancers.[5] This spectrum of differing efficacy has been most clearly demonstrated in the β-carbolines[4,6] **1** and, more recently, in the imidazobenzodiazepine series, **2**.[7,8]

[†] Ferrosan Research Division.
[‡] Chemistry Department, Merck Sharp and Dohme Research Laboratories.
[§] Biochemistry Department, Merck Sharp and Dohme Research Laboratories.
[∥] Merck Sharp and Dohme Research Laboratories, Rahway, NJ.

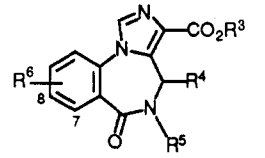
(1) Olsen, R. W. *Annu. Rev. Pharmacol. Toxicol.* **1982**, *22*, 245.
(2) Martin, I. L. *TIPS* **1984**, *5*, 343.
(3) Haefely, W.; Kyburz, E.; Gerecke, M.; Mohler, H. *Adv. Drug Res.* **1985**, *14*, 165.
(4) Braestrup, C.; Honore, T.; Nielsen, M.; Petersen, E. N.; Jensen, L. H. *Biochem. Pharmacol.* **1984**, *33*, 859.
(5) Vernault, P.; Chapouthier, G.; Prado de Carvalho, L.; Simiand, J.; Mone, M.; Dodd, R. H.; Rossier, J. *Nature* **1986**, *321*, 864.
(6) Braestrup, C.; Schmiechen, R.; Neef, G.; Nielsen, M.; Petersen, E. N. *Science* **1982**, *216*, 1241.
(7) Haefely, W. *Clin. Neuropharmacol.* **1984**, *7* (Suppl. 1), 5363.
(8) Kemp, J. A.; Marshall, G. R.; Wong, E. H. F.; Woodruff, G. N. *Br. J. Pharmacol.* **1987**, *91*, 601.

0022-2623/89/1832-2282$01.50/0



1

	a	b	c	d	e
R^3	C_2H_5	C_2H_5	C_2H_5	C_2H_5	CH_3
R^4	CH_2OCH_3	CH_2OCH_3	CH_3	H	C_2H_5
R^5	H	OCH_2Ph	$OCH(CH_3)_2$	H	H
R^6	OCH_2Ph	H	H	H	OCH_3
R^7	H	H	H	H	OCH_3
	full agonist	partial agonist	antagonist	partial inverse agonist	full inverse agonist



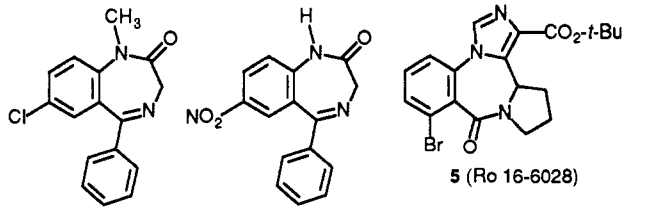
2a: (Ro 15-1788): $R^3 = C_2H_5$; $R^4 = H$; $R^5 = CH_3$; $R^6 = 8\text{-F}$
b: $R^3 = C_2H_5$; $R^4 = H$; $R^5 = CH_3$; $R^6 = 7\text{-Cl}$

Classical full agonists (such as diazepam (**3**) and nitrazepam (**4**)) at the benzodiazepine receptor continue to be widely prescribed as anxiolytics, anticonvulsants, sedatives, and muscle relaxants. These latter two properties, however, are often seen as unwanted side effects and are believed to be related to the high level of receptor stimulation achieved by full agonists. Partial agonists are of interest since they are proposed to be capable of selectively eliciting the lower efficacy responses such as anxiolysis.[9]

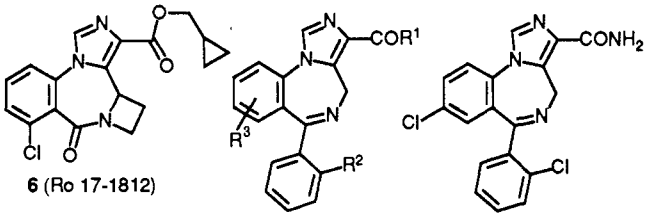
Several carboxylic acid derivatives of the imidazobenzodiazepines (e.g., **5–8**) have been reported to act as partial agonists at the benzodiazepine receptor; at least three (Ro 16-6028, **5**; Ro 17-1812, **6**; and Ro 23-0364, **7a**) have been evaluated in humans.[10] In the β-carbolines, it has been demonstrated[11] that replacement of the carboxyl derivative at the 3-position by a 1,2,4-oxadiazole moiety led to a series of highly active benzodiazepine receptor ligands. Furthermore, this replacement was found to give rise to compounds with higher intrinsic efficacy as compared to the corresponding ethyl esters. Major chemical similarities exist between the imidazobenzodiazepine framework represented by the benzodiazepine receptor antagonist Ro 15-1788 (**2a**) and the β-carboline framework represented by **1d** (Figure 1). It was anticipated therefore that this substitution in both the 6-oxo- and 6-aryl-imidazobenzodiazepines should provide novel partial agonists with a favorable separation between anxiolytic and sedative properties. Accordingly, this paper describes the synthesis, receptor-binding properties, and conformational features of molecules generically described by **9–12**.

Results

I. Synthetic Chemistry. Compounds listed in Tables I and II were made by one of three routes starting from

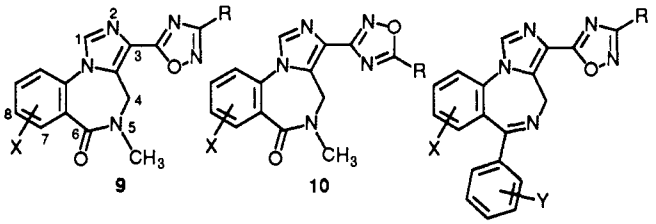


3 (diazepam) **4** (nitrazepam) **5** (Ro 16-6028)

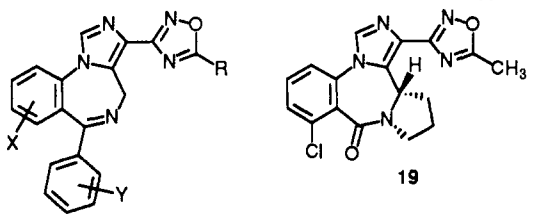


6 (Ro 17-1812)

7a: $R^1 = NH_2$; $R^2 = Cl$; $R^3 = H$ **8** (Ro 21-8384)
b: $R^1 = OC_4H_9^t$; $R^2 = H$; $R^3 = 7\text{-Cl}$



9 **10** **11**

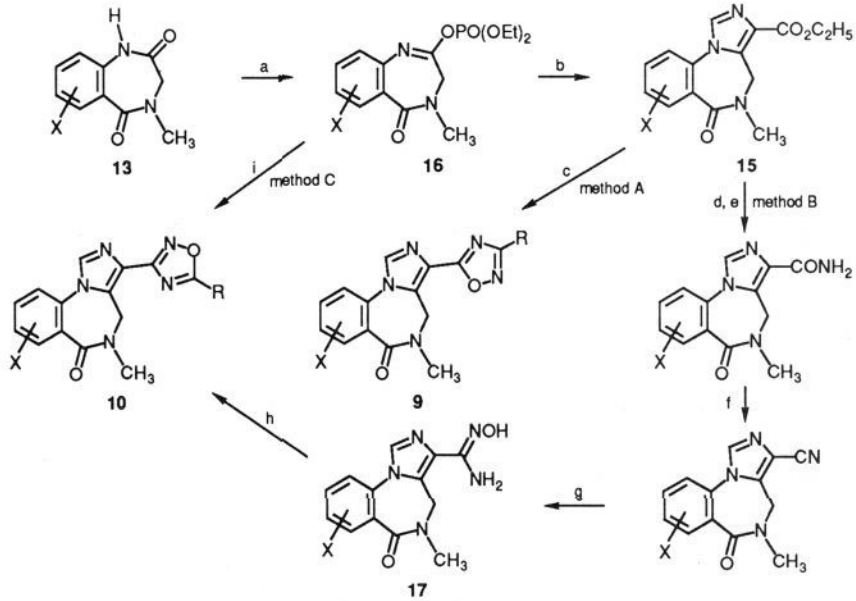


12 **19**

dione **13** for the 6-oxo series (Scheme I) and from lactam **14** for the 6-aryl series (Scheme II). These starting materials were available via chemistry[12,13] from the appropriate δ-aminobenzoic acids and 2-aminobenzophenones, respectively. Preparation of ester **15** followed the published procedures[12] by generation of phosphate ester **16**. This reactive intermediate was not isolated but treated immediately with the anion derived from ethyl isocyanoacetate and potassium *tert*-butoxide or LDA to afford the imidazo ester **15**. 3-Alkyl-1,2,4-oxadiazoles **9** were available directly from this ester by reaction with the appropriate alkyl amide oxime under basic conditions. Initially, synthesis of 5-alkyl-1,2,4-oxadiazoles was achieved by a circuitous route requiring elaboration of the ester group in **15** to the amide oxime **17** as shown. Treatment of **17** with a carboxylic acid anhydride accomplished final ring closure to oxadiazole **10**. A more convergent procedure was subsequently developed involving the intermediacy of 3-(isocyanomethyl)oxadiazole **18** (Scheme III). By using exactly the same conditions employed in the preparation of **15**, 5-alkyloxadiazole **10** was available in one step from dione **13**. Close examination of the reaction revealed that the isonitrile anion was extremely sensitive and rapidly decomposed on warming. Use of a much lower reaction temperature (−70 °C) and LDA as the base allowed the anion to be more reliably produced with concomitant im-

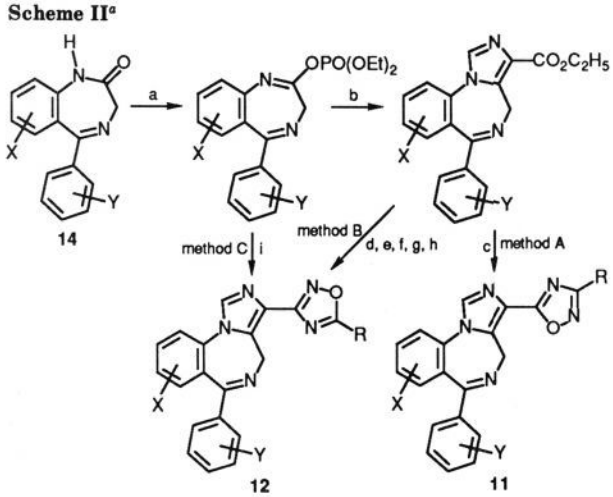
(9) Petersen, E. N.; Jensen, L. H.; Diejer, J.; Honore, T. *Pharmacopsychiatry* **1986**, *19*, 4–6.
(10) Mertz, W. A.; et al. Abstracts from IV World Congress of Biological Psychiatry, Sept 1985, Philadelphia, Raven Press: New York, p 408. Haefely W. *Clin. Neuropharmacol.* **1984**, *7* (Suppl. 1), 670.
(11) Bondo Hansen, J.; Engelstoft, M.; Honore, T.; Watjon Soebrog, F.; Schmiechen, R., Berlin (West) P.2, 10, 13, 3-(1,2,4-oxadiazolyl)-β-carboline derivatives as benzodiazepine receptor ligands. Data presented at the IXth International Symposium on Medicinal Chemistry, Bêrlin, Sept 14–18, 1986.
(12) The compounds are disclosed in a series of patents to Roche. A leading reference is: Gerecke, M.; Haefely, W.; Kyburz, E.; Mohler, H.; Pieri, L.; Polc, P. U.S. Patent, 4,346,031, 1982.
(13) Walser, A.; Fryer, R. I. U.S. Patent 4,280,957, 1981. Sternback, L. H.; Reeder, E. U.S. Patent 3,136,815, 1964.

Scheme I[a]



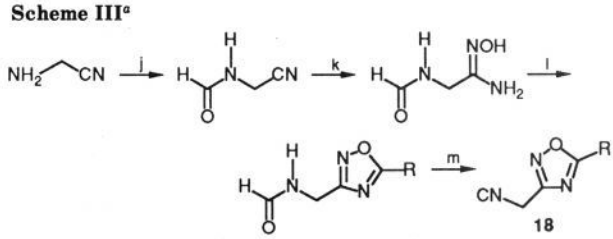
[a] Reagents: (a) NaH, DMF, $ClPO(OEt)_2$; (b) $CNCH_2CO_2C_2H_5$, $KOBu^t$, DMF (or LDA, THF); (c) Na, EtOH, molecular sieve, RC(=NOH)NH_2; (d) 2 N NaOH then concentrated HCl; (e) Et_3N, $ClCO_2Et$, CH_2Cl_2 then NH_3 gas; (f) Et_3N, TFAA, dioxane; (g) NH_2OH·HCl, K_2CO_3, H_2O, EtOH; (h) $(RCO)_2O$ or RCO_2CH_3; (i) LDA, THF, 18.

Scheme II[a]



[a] Reagents: see footnotes to Scheme I.

Scheme III[a]



[a] Reagents: (j) $HC(OCH_3)$; (k) NH_2OH·HCl, $NaOCH_3$, MeOH; (l) $RCO_2CH_3(C_2H_5)$, Na, EtOH; (m) $POCl_3$, Et_3N, CH_2Cl_2 then Na_2CO_3.

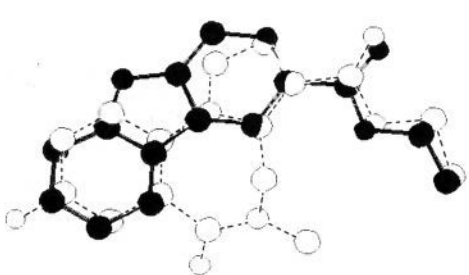


Figure 1. Comparison of benzodiazepine **2a** with a betacarboline (**1d**), both antagonists at the benzodiazepine receptor.

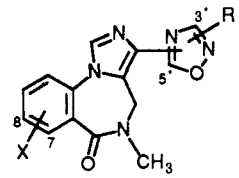
provement in the overall conversion to the required imidazole (40–60% yield). Similar methodology (Scheme II) gave access to the 6-aryl series **11** and **12**.

II. Biochemistry. The affinity of each of the new compounds **9–12** for the benzodiazepine receptor in rat cortical membranes was measured by its ability to displace the specific binding of the radiolabeled antagonist [^{3}H]-Ro 15-1788[14] (Tables I and II). In addition, an assessment of the efficacy of these compounds was made by measuring the improvement in their affinity in response to $GABA_A$-receptor activation (the well-known "GABA shift") following addition of a fixed concentration of GABA to the assay.[15] Without exception, each compound displaced the radioligand with a mass action profile and a Hill coefficient close to unity consistent with the recognition of a uniform population of sites.

III. X-ray Crystallography. A structurally related pair of compounds, one from the 6-oxo series (**10h**) and one from the 6-aryl series (**12o**), were studied by X-ray crystallography (Figure 2) so that a direct comparison could be made with the crystallographically determined structure of Ro-15 1788[16] (**2a**, Figure 3A). It was discovered that **10h** could be crystallized as two distinct polymorphs; the first was isolated after crystallization from ethyl acetate (**10h–I**, mp 165 °C) and the second from acetone (**10h-II**, mp 135 °C). **10h-I** (Figure 3B) had only a single molecule in the asymmetric unit while **10h-II** had two molecules (**10h-IIa** and **10h-IIb**, Figure 3C–D) in

(14) Mohler, H.; Burkard, N. P.; Keller, H. H.; Richards, J. G.; Haefely, W. *J. Neurochem.* **1981**, *37*, 714.
(15) Ehlert, F. J.; Roeske, W. R.; Gee, K. W.; Yamamura, H. I. *Biochem. Pharmacol.* **1983**, *32*, 2375.
(16) Codding, P. W.; Muir, A. K. S. *Mol. Pharmacol.* **1985**, *28*, 178.

Table I. Summary of Method of Preparation and Physical Properties and in Vitro Binding Data for Compounds **9** and **10**



no.	method[a]	X	R	recryst solvent (% yield)	mp, °C	formula	IC_{50},[b] nM (SEM)	GABA shift (SEM)[c]
9a	1A	H	3-CH_3	EtOH/H_2O (25)	253–254	$C_{15}H_{13}N_5O_2$	67	1.50
9b	1A	H	3-C_2H_5	*i*-PrOH (39)	191–192	$C_{16}H_{15}N_5O_2$	51 ± 9	1.79 ± 0.17
9c	1A	H	3-*n*-C_3H_7	EtOH (40)	158–159	$C_{17}H_{17}N_5O_2$	120	2.08
9d	1A	H	3-*i*-CH_3H_7	EtOH/H_2O (45)	202–203	$C_{17}H_{17}N_5O_2$	105	2.19
10a	1C	H	5-CH_3	DMF/H_2O (12)	263–264	$C_{15}H_{13}N_5O_2$	27 ± 4	1.28 ± 0.13
10b	1B	H	5-C_2H_5	*d* (19)	204–206	$C_{16}H_{15}N_5O_2$	22 ± 1	1.80 ± 0.06
10c	18	H	5-*n*-C_3H_7	MeOH/CH_2Cl_2 (55)	146–147	$C_{17}H_{17}N_5O_2$	34	2.0
10d	1B	H	5-*i*-C_3H_7	*i*-PrOH (25)	172–175	$C_{17}H_{17}N_5O_2$·0.2i-PrOH	53	2.4
9e	1A	8-Cl	3-C_2H_5	*d* (40)	169–179	$C_{16}H_{14}ClN_5O_2$	600	0.91
9f	1A	7-Cl	3-CH_3	EtOH/H_2O (35)	208–210	$C_{15}H_{15}ClN_5O_2$	8.1 ± 2.0	1.51 ± 0.13
9g	1A	7-Cl	3-C_2H_5	EtOH/H_2O (40)	184–186	$C_{16}H_{14}ClN_5O_2$	9.7 ± 0.2	1.43 ± 0.13
9h	1A	7-Cl	3-*n*-C_3H_7	EtOH/H_2O (80)	172–174	$C_{17}H_{16}ClN_5O_2$	6	1.53
9i	1A	7-Cl	3-*i*-C_3H_7	EtOH/H_2O (89)	158–160	$C_{17}H_{16}ClN_5O_2$	14 ± 0.6	1.86 ± 0.09
10e	1C	7-Cl	5-CH_3	EtOAc (30)	289–291	$C_{15}H_{12}ClN_5O_2$	3.4 ± 0.3	1.18 ± 0.13
10f	1B	7-Cl	5-C_2H_5	*i*-PrOH (50)	227–229	$C_{16}H_{14}ClN_5O_2$	6.4	1.12
10g	1C	7-Cl	5-*n*-C_3H_7	EtOAc (15)	171–172	$C_{17}H_{16}ClN_5O_2$	3.8	1.41
10h	1C	7-Cl	5-*i*-C_3H_7	*d* (44)	146–147	$C_{17}H_{16}ClN_5O_2$	3.6 ± 0.4	2.37 ± 0.13
9j	1A	7-F	3-CH_3	EtOH/H_2O (17)	146–148	$C_{15}H_{12}FN_5O_2$	22 ± 8	1.85 ± 0.14
9k	1A	7-F	3-C_2H_5	*i*-PrOH (35)	207–208	$C_{16}H_{14}FN_5O_2$	42	2.4
9l	1A	7-F	3-*n*-C_3H_7	*i*-PrOH (45)	163–164	$C_{17}H_{16}FN_5O_2$	24 ± 3	1.94 ± 0.18
9m	1A	7-F	3-*i*-C_3H_7	EtOH/H_2O (49)	189–190	$C_{17}H_{16}FN_5O_2$	67	2.31
10i	1C	7-F	5-CH_3	EtOAc (15)	250–253	$C_{15}H_{12}FN_5O_2$	11 ± 0.7	1.65 ± 0.15
10j	1C	7-F	5-C_2H_5	EtOAc (21)	215–216	$C_{16}H_{14}FN_5O_2$	15 ± 5	1.44 ± 0.14
10k	1C	7-F	5-*n*-C_3H_7	EtOAc (18)	275–275	$C_{17}H_{16}FN_5O_2$	14 ± 7	1.70 ± 0.19
10l	1C	7-F	5-*i*-C_3H_7	EtOAc (20)	181–183	$C_{17}H_{16}FN_5O_2$	19 ± 8	1.97 ± 0.24
9n	1A	8-F	3-CH_3	EtOAc/Et_2O (25)	238	$C_{15}H_{12}FN_5O_2$	50	1.47
9o	1A	8-F	3-C_2H_5	*d* (29)	183–184	$C_{16}H_{14}FN_5O_2$·0.5H_2O	105	2.34
9p	1A	8-F	3-*n*-C_3H_7	*i*-PrOH (7)	115–116	$C_{17}H_{16}FN_5O_2$	42	2.0
9q	1A	8-F	3-*i*-C_3H_7	*i*-PrOH (17)	192	$C_{17}H_{16}FN_5O_2$	100	1.86
10m	1C	8-F	5-CH_3	CH_2Cl_2/Et_2O (11)	234–236	$C_{15}H_{12}FN_5O_2$	21	1.53
10n	1C	8-F	5-C_2H_5	CH_2Cl_2/Et_2O (15)	189–190	$C_{16}H_{14}FN_5O_2$	27	1.84
10o	1C	8-F	5-*n*-C_3H_7	CH_2Cl_2/Et_2O (21)	127–128	$C_{17}H_{16}FN_5O_2$	27	1.96
10p	1C	8-F	5-*i*-C_3H_7	CH_2Cl_2/Et_2O (25)	179–180	$C_{17}H_{16}FN_5O_2$	43	2.76
2a (Ro15-1788)							3.8 ± 1.0	1.04 ± 0.09
3 (diazepam)							77 ± 12	3.07 ± 0.09
5 (Ro16-6028)							0.6 ± 0.2	1.34 ± 0.13
6 (Ro17-1812)							4.2 ± 0.8	1.70 ± 0.08

[a] See the Experimental Section for general procedures. Data for standard benzodiazepine ligands also included for comparison. [b] Binding affinities in rat cortical membranes as described in the Experimental Section; SEM = standard error of the mean from $n \geq 3$. Where SEM is not quoted, the figures are the mean of two independent determinations typically with individual values within ±25% of the mean. [c] Ratio of the binding affinity in the presence (0.3 mM) and absence of GABA. [d] Compound isolated pure directly from chromatography on silica.

different conformations as well as a single molecule of acetone.[17] The conformations of the imidazobenzodiazepine portions of all three molecules were mutually identical. The principle difference among the molecules was the dihedral angle between the imidazole and oxadiazole rings (N13–C14–C15–N16, Table III). The conformation of the diazepine ring in **12o** (Figure 3E) was essentially the same as that in **10h** and also diazepam. The phenyl ring was twisted relative to the diazepine ring so that the dihedral angle C11–C5–C23–C28 was 34° compared to 26° for diazepam.[19] A complete comparison of important geometrical variables is given in Table III.

IV. Discussion. A. SAR and Comparison with Imidazobenzodiazepine Esters. It has already been established[3] in the imidazobenzodiazepine-3-carboxylic acid ester series, **2**, related to **9** and **10** that there is a wide tolerance in the size of alkyl group that is acceptable with little or no effect on binding. Since the oxadiazole system is seen as an isosteric replacement for the ester linkage, it was not unexpected that variation of the 3- or 5-alkyl substituent only marginally altered the affinity of the ligand for the receptor (for numbering, see Tables I and II). Surprisingly, however, this same substituent had a significant effect on efficacy (as measured by the GABA shift) with the trend usually being toward increased efficacy with increasing bulk in the vicinity of the oxadiazole ring. All compounds, however, with the exception of the relatively inactive derivative **9e**, had efficacies in the partial agonist range. As predicted from earlier experience in the

(17) For **10h** and **12o**, molecular models were constructed directly from the X-ray data by using the Merck Molecular Modelling Software, MOLEDIT. These models and the coordinates of **2a** were read into the CHEMX program for further analysis. CHEMX software was obtained from Chemical Design Ltd., Oxford, United Kingdom. In the subsequent discussion of the structural properties of these molecules, the numbering scheme derived from the X-ray crystal structures is adopted.

(18) Camerman, A.; Camerman, N. *J. Am. Chem. Soc.* **1972**, *94*, 268.

(19) Tebib, S.; Bourguignon, J. J.; Wermuth, C. G. *J. Computer-Aided Mol. Design* **1987**, *1*, 153.

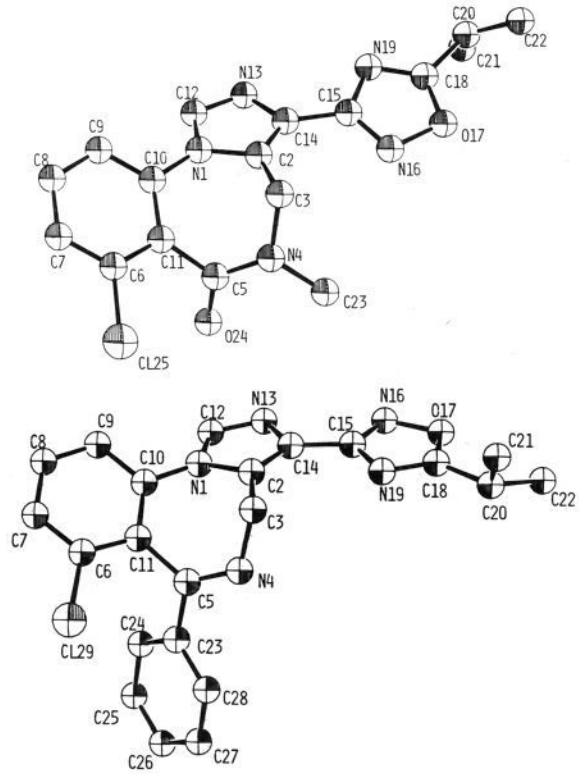


Figure 2. Perspective drawings of (A) **10h-I** and (B) **12o** generated by computer from the final X-ray coordinates.

β-carboline series, both oxadiazole analogues of ester **2a** (**9o** and **10n**) are more efficacious than the starting ester although considerably less potent. It will become obvious below that this change in affinity is more associated with the detrimental effect of substitution at the 8-position in **9** and **10** rather than the influence of the oxadiazole ring. The 5-alkyloxadiazole series were normally at least 2-fold more potent than the 3-alkyl analogues but with no consistent trend in GABA shift.

The most significant effect on activity and a clear divergence of SAR between the ester and oxadiazole series were changes to the substitution pattern of the benzo ring. In the ethyl ester series **2** (R^3 = Et; R^4 = H; R^5 = CH_3) no enhancement in affinity was achieved by 7-halo or 8-fluoro substitution. Shifting the chlorine atom to position 8 reduced affinity 2-fold in clear contrast to the rules observed for classical agonists such as diazepam. The position of the halogen substituent also moderated efficacy since the 7-chloro derivative was shown to be an inverse agonist. In the current range of oxadiazoles, a 7-halo substituent invariably caused an increase in receptor affinity (with the effect being most pronounced for chloro where a maximally 10-fold improvement was found) relative to the unsubstituted (**9a–d**, **10a–d**) or 8-halo compounds. As with the esters, the 7-chloro derivatives were the least efficacious ligands although all maintained some level of partial agonist behavior. Compound **10f** provided the ligand most closely resembling a receptor antagonist.

Little information has been published regarding SAR in the 6-aryl series of imidazobenzodiazepine esters **7**. The *tert*-butyl ester **7b** had diazepam-like affinity but low efficacy while the 8-chloro esters were partial agonists. Obvious trends for the activities of **11** and **12** were far less distinct than those observed for **9** and **10**: 5-Alkyl derivatives were usually more potent than the 3-alkyl cogener; a 7-halo substituent invariably enhanced whereas 8-sub-

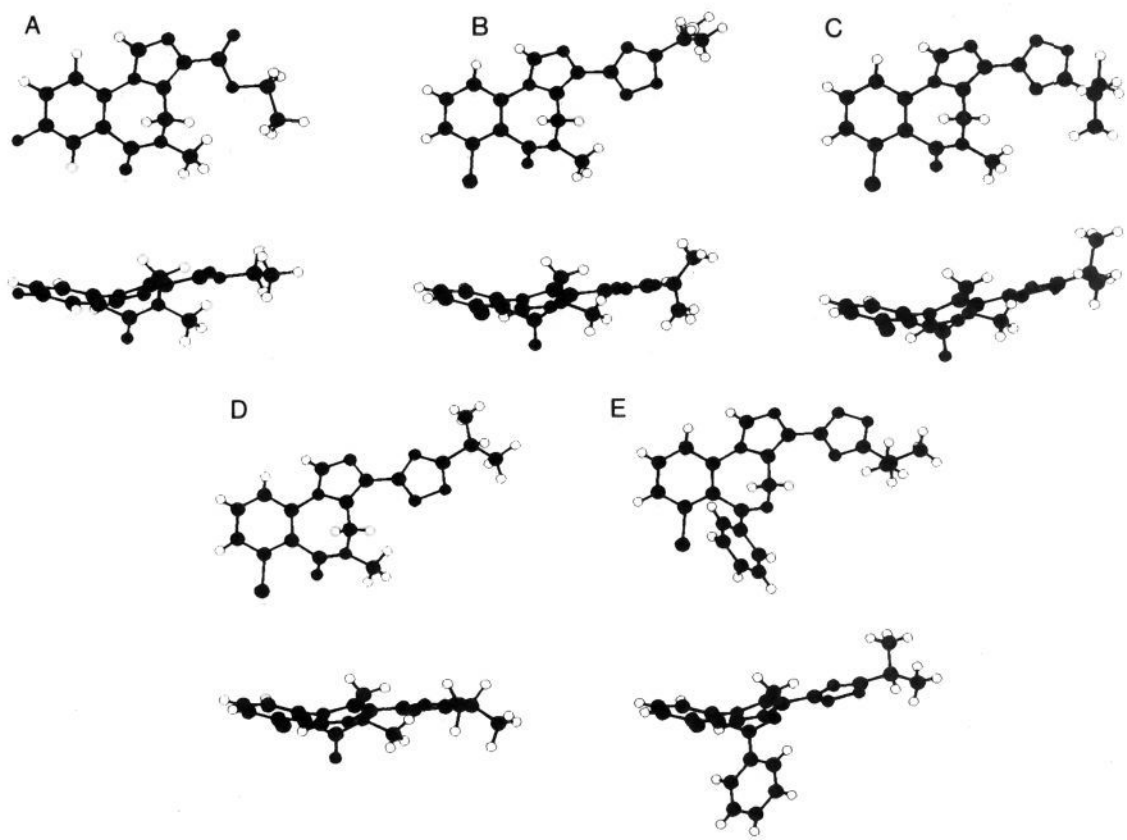
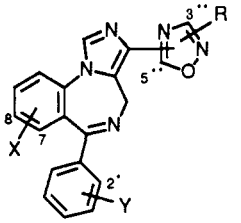


Figure 3. Comparison of the X-ray structures of **10h** and **12o** with Ro 15-1788 (**2a**). The benzodiazepine rings of each structure were superimposed ("FLY" in CHEMX) so that orthogonal views of each are presented in the same orientation: A, **2a**; B, **10h-I**; C, **10h-IIa**; D, **10h-IIb**; E, **12o**.

Table II. Summary of Method of Preparation, Physical Properties, and in Vitro Binding Data for Compounds 11 and 12



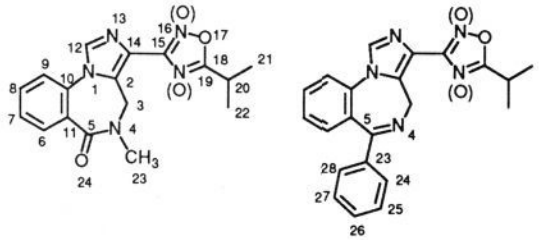
no.	method[a]	X	Y	R	recryst solvent (% yield)	mp, °C	formula	IC_{50},[b] nm (SEM)	GABA shift (SEM)[c]
11a	2A	H	H	3-CH_3	EtOH/H_2O (73)	179–180	$C_{20}H_{15}N_5O$	24 ± 3	1.94 ± 0.18
11b	2A	H	H	3-C_2H_5	*d* (80)	183	$C_{21}H_{17}N_5O{\cdot}0.67H_2O$	19	1.2
11c	2A	H	H	3-*n*-C_3H_7	CH_2Cl_2/Et_2O (30)	116	$C_{22}H_{19}N_5O$	43	1.8
11d	2A	H	H	3-*i*-C_3H_7	CH_2Cl_2/Et_2O (75)	169	$C_{22}H_{19}N_5O$	52	1.7
12a	2B	H	H	5-CH_3	EtOA (31)	183–185	$C_{20}H_{15}N_5O$	7.0 ± 1.5	1.56 ± 0.06
12b	2B	H	H	5-C_2H_5	EtOAc (40)	184–185	$C_{21}H_{17}N_5O{\cdot}0.2H_2O$	16 ± 2.6	2.11 ± 0.38
12c	2B	H	H	5-*i*-C_3H_7	EtOAc (45)	124–126	$C_{22}H_{19}N_5O{\cdot}H_2O$	18	2.57
11e	2A	8-Cl	H	3-CH_3	CH_2Cl_2/Et_2O (15)	272–272.5	$C_{20}H_{14}ClN_5O$	180	1.84
11f	2A	8-Cl	H	3-C_2H_5	CH_2Cl_2/Et_2O (41)	214.5–215.5	$C_{21}H_{16}ClN_5O{\cdot}0.1H_2O$	160	2.0
11g	2A	8-Cl	H	3-*n*-C_3H_7	d(39)	114–115	$C_{22}H_{18}ClN_5O$	120	2.57
11h	2A	8-Cl	H	3-*i*-C_3H_7	CH_2Cl_2/Et_2O (47)	155	$C_{22}H_{18}ClN_5O$	120	1.06
12d	2C	8-Cl	H	5-CH_3	*d* (18)	261.5–363.5	$C_{20}H_{14}ClN_5O$	71	1.17
12e	2C	8-Cl	H	5-C_2H_5	*d* (17)	215–216	$C_{21}H_{16}ClN_5O$	44	2.43
12f	2C	8-Cl	H	5-*n*-C_3H_7	*d* (15)	145–146	$C_{22}H_{18}ClN_5O$	45	2.22
12g	2C	8-Cl	H	5-*i*-C_3H_7	*d* (15)	158–163	$C_{22}H_{18}ClN_5O$	53	2.89
12j	2C	8-Cl	2′-Cl	5-*n*-C_3H_7	CH_2Cl_2/Et_2O (45)	172	$C_{22}H_{17}Cl_2N_5O$	8.2 ± 0.8	1.81 ± 0.14
12k	2C	8-Cl	2′-Cl	5-*i*-C_3H_7	CH_2Cl_2/Et_2O (50)	227	$C_{22}H_{17}Cl_2N_5O$	12 ± 1.0	2.79 ± 0.28
11m	2A	7-Cl	H	3-CH_3	*d* (23)	276–277	$C_{20}H_{14}ClN_5O$	3.4 ± 0.1	1.67 ± 0.17
11n	2A	7-Cl	H	3-C_2H_5	*d* (45)	235	$C_{21}H_{16}ClN_5O$	4.1 ± 0.4	2.21 ± 0.23
11o	2A	7-Cl	H	3-*n*-C_3H_7	*d* (45)	212	$C_{22}H_{18}ClN_5O$	5.2 ± 0.5	1.85 ± 0.11
11p	2A	7-Cl	H	3-*i*-C_3H_7	*d* (45)	251–252	$C_{22}H_{18}ClN_5O{\cdot}0.1H_2O$	7.2	1.91
12l	2C	7-Cl	H	5-CH_3	*d* (52)	268–269	$C_{20}H_{14}ClN_5O$	1.3 ± 0.2	1.44 ± 0.22
12m	2C	7-Cl	H	5-C_2H_5	CH_2Cl_2/Et_2O (55)	255–256	$C_{22}H_{16}ClN_5O$	2.8 ± 0.4	1.65 ± 0.11
12n	2C	7-Cl	H	5-*n*-C_3H_7	CH_2Cl_2/Et_2O (33)	226–227	$C_{22}H_{18}ClN_5O$	3.9 ± 1.0	1.70 ± 0.20
11i	2A	8-Cl	2′-Cl	3-CH_3	CH_2Cl_2/Et_2O (25)	192	$C_{20}H_{13}Cl_2N_5O$	110	2.28
11j	2A	8-Cl	2′-Cl	3-C_2H_5	CH_2Cl_2/Et_2O (31)	216–217	$C_{21}H_{15}Cl_2N_5O$	25 ± 4	2.11 ± 0.53
11k	2A	8-Cl	2′-Cl	3-*n*-C_3H_7	*d* (30)	185–186	$C_{22}H_{17}Cl_2N_5O$	34	2.92
11l	2A	8-Cl	2′-Cl	3-*i*-C_3H_7	CH_2Cl_2/Et_2O (60)	227–228	$C_{22}H_{17}Cl_2N_5O$	45	1.81
12h	2C	8-Cl	2′-Cl	5-CH_3	Et_2O (30)	225–226	$C_{20}H_{13}Cl_2N_5O$	20	1.83
12i	2C	8-Cl	2′-Cl	5-C_2H_5	CH_2Cl_2/Et_2O (20)	245–246	$C_{21}H_{15}Cl_2N_5O$	9.4 ± 1.6	1.86 ± 0.12
12o	2C	7-Cl	H	5-*i*-C_3H_7	*d* (38)	265–266	$C_{22}H_{18}ClN_5O$	6.9 ± 2.7	1.82 ± 0.29
11q	2A	H	2′-Cl	3-CH_3	CH_2Cl_2/Et_2O (25)	238–239	$C_{20}H_{14}ClN_5O$	31	1.29
11r	2A	H	2′-Cl	3-*i*-C_3H_7	CH_2Cl_2/Et_2O (35)	218–219	$C_{22}H_{18}ClN_5O$	31	1.83
12p	2C	H	2′-Cl	5-CH_3	CH_2Cl_2/Et_2O (35)	230–232	$C_{20}H_{14}ClN_5O$	6.7	1.35
12q	2C	H	2′-Cl	5-C_2H_5	*d* (30)	222–223	$C_{21}H_{16}ClN_5O$	13	1.48
12r	2C	H	2′-Cl	5-*n*-C_3H_7	*d* (32)	191–192	$C_{22}H_{18}ClN_5O{\cdot}0.5H_2O$	12	1.68
12s	2C	H	2′-Cl	5-*i*-C_3H_7	CH_2Cl_2/Et_2O (20)	218–219	$C_{22}H_{18}ClN_5O$	14	1.64
11s	2A	7-F	H	3-CH_3	CH_2Cl_2/Et_2O (18)	242–244	$C_{20}H_{14}FN_5O{\cdot}0.15H_2O$	8.3 ± 1.5	1.84 ± 0.31
11t	2A	7-F	H	3-C_2H_5	CH_2Cl_2/Et_2O (20)	223–224	$C_{21}H_{16}FN_5O$	9.8 ± 1.4	2.31 ± 0.35
11u	2A	7-F	H	3-*n*-C_3H_7	CH_2Cl_2/Et_2O (25)/hexane	154–155	$C_{22}H_{18}FN_5O$		
11v	2A	7-F	H	3-*i*-C_3H_7	CH_2Cl_2/Et_2O (23)	239–240	$C_{22}H_{18}FN_5O$	1.4 ± 2.4	2.03 ± 0.28
12t	2C	7-F	H	5-CH_3	CH_2Cl_2/Et_2O (15)	215–216	$C_{20}H_{14}FN_5O$	2.4 ± 0.4	1.68 ± 0.16
12u	2C	7-F	H	5-C_2H_5	CH_2Cl_2/Et_2O (60)	222–224	$C_{21}H_{16}FN_5O{\cdot}0.1H_2O$	7.5 ± 2.0	1.82 ± 0.11
12v	2C	7-F	H	5-*n*-C_3H_7	CH_2Cl_2/Et_2O (22)	186–187	$C_{22}H_{18}FN_5O{\cdot}0.5H_2O$	5.3 ± 0.7	2.06 ± 0.07
12w	2C	7-F	H	5-*i*-C_3H_7	CH_2Cl_2/Et_2O (27)	262–263	$C_{22}H_{18}FN_5O$	6.1 ± 094	2.08 ± 0.14

[a–d] See footnotes to Table I.

stitution diminished receptor affinity relative to the unsubstituted compound; there were no consistent patterns in variability of the GABA shift. In the more potent subsets having a 7-halo substituent, the 6-aryl derivatives (**11** and **12**) were uniformally more potent (up to 5-fold) and more efficacious than the 6-oxo counterparts. In summary, the predominant biochemical differences that characterize replacement of the ester functionality by an oxadiazole ring in these series of imidazobenzodiazepines are (a) increased partial agonist properties and (b) affinity for the benzodiazepine receptor which is accentuated by a 7-halo substituent.

B. The Benzodiazepine Receptor Model. It is informative to speculate how the findings of the present study can be accommodated by the structural models proposed for the binding of benzodiazepine ligands to their receptor. The most recently published model[19] attempted to unify SAR of ligands not only from widely different structural classes but also with efficacies spanning the entire efficacy range from inverse agonists through antagonists to full agonists. We have chosen to discuss the structural properties of compounds **9–12** in terms of the model proposed by Codding and Muir[16] based on the X-ray structure of **2a** and other benzodiazepine ligands because of the 3-dimensional similarity of the respective compounds. This model involved six possible interactions to account for both agonist and antagonist behavior of benzodiazepine-based ligands, the key features of which are summarized in Figure 4. It is clear from Figure 3 and the geometry variables listed in Table III that the gross molecular structure of the imidazobenzodiazepine framework remains unaltered in the various crystal forms of **10h** and

Table III. Comparison of Important Geometric Variables in the X-ray Crystal Structures of **2a**, **10h**, and **12o**[a]



geometry variable[b]	2a	10h-I	10h-IIa	10h-IIb	12o
(a) Planes (Interior Angles), deg					
Pla1–Pla2	147.6	142.3	138.7	147.2	144.2
Pla2–Pla3	123.8	127.4	126.9	126.0	123.0
Pla2–Pla4	151.0	141.9	140.3	143.2	143.6
Pla4–Pla5	18.0	20.1	15.3	14.2	5.5
Pla1–Pla4	145.2	141.9	134.3	143.2	140.7
(b) Dihedral Angles, deg					
N1–C10–C11–C5	−9.3	−7.7	−4.8	−7.3	−6.8
N13–C14–C15–N(O)16[c]	−161.0	−158.2	14.8	−163.6	−0.1
(c) Distances, Å					
Cen1–N1	2.81	2.80	2.79	2.79	2.80
Cen1–N4	3.81	3.80	3.77	3.82	3.82
Cen1–O15	3.60	3.64	3.67	3.64	
Cen1–N13	4.94	4.95	4.94	4.93	4.95
Cen1–N(O)16	7.30	6.73	7.31	6.73	7.33
Cen1–N(O)19	6.60	7.38	6.73	7.40	6.72

[a] Coordinated for each structure read into CHEMX[18] and geometry measured by using the "FLY" option. [b] Definition of planes and centroid as follows: Cen1 and Pla1, C6–C7–C8–C9–C10–C11; Pla2, N1–C2–N4–N5; Pla3, C2–C3–N4; Pla4, N1–C2–C14–N13–C12; Pla5, C15–N(O)16–N(O)19. For numbering scheme, see diagram at top of table. [c] For **2a**, carbonyl oxygen is assigned O23 and ether oxygen is O26.

12o relative to **2a**. The separation between the center of the benzo ring (1 in Figure 4) and hydrogen bond acceptor group (2) is often regarded[20] as a predictor of efficacy for a given molecule: for agonists such as diazepam the separation is 4.91 Å whereas for the antagonist **2a** it is 7.30 Å if the ester carbonyl is the candidate for this site. Alternatively, the imidazole nitrogen (N-13, crystal structure numbering) may be allocated the same role but this would not allow the model to distinguish agonists and antagonists since the separation of this moiety from the benzo ring is 4.95 Å. It is assumed that either of the oxadiazole ring nitrogen atoms function in the same way as the ester oxygens. Since the separation between 1 and either contender for 2 in the oxadiazole-based partial agonists **10h** and **12o** is identical with that in the antagonist **2a**, other more subtle structural properties must also contribute to the level of efficacy. One possibility is that the choice of acceptor site that is recognized by the receptor (i.e. either imidazole-ring nitrogen or ester/oxadiazole) is governed by minor structural and electronic factors that may influence the relative strength of the hydrogen-bonding interaction.

To explore this possibility further, partial atomic changes were calculated by using the MOPAC method[21] for the oxadiazole **10h** and the corresponding ester **2b**. In order to allow maximum overlap of the hydrophobic region (3), the rotamer of the oxadiazole represented by **10h-IIa** (Figure 3) was used in the calculation, and the results are shown in Figure 5. In the case of ester **2b**, the partial charge residing on the carbonyl oxygen was significantly

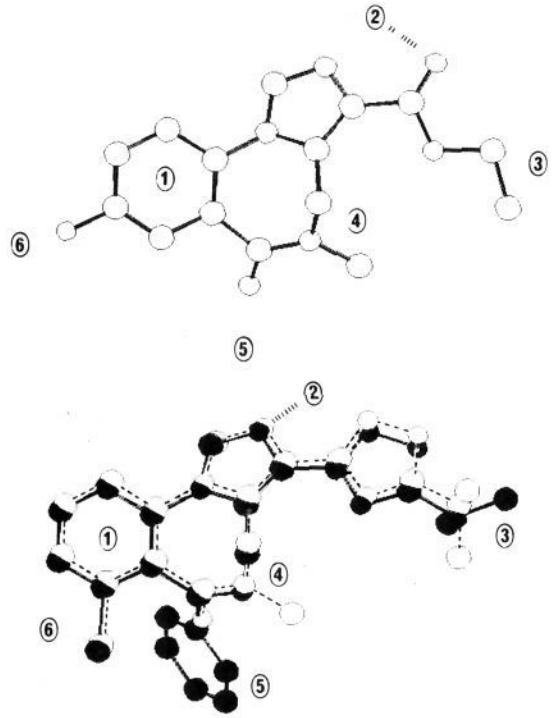


Figure 4. A. Recognition sites for the benzodiazepine receptor model as proposed by Codding and Muir[17] simplified to describe the binding of ligands having a benzodiazepine nucleus. Antagonists are assumed to rely predominantly on interactions 1–3 (a benzo ring, a hydrogen-bond acceptor site, and a hydrophobic side chain, respectively) with the acceptor site 2 being localized toward the ester carbonyl oxygen. An additional three features necessary to account for agonist binding are represented by 4–6 (diazepine ring imine nitrogen; a phenyl ring not present in **2a** and an electronegative 8-substituent). B. Modified model to describe binding mode of oxadiazoles **10h** (open circles) and **12o** (solid circles and bonds). The two molecules were superimposed and then slightly offset for clarity.

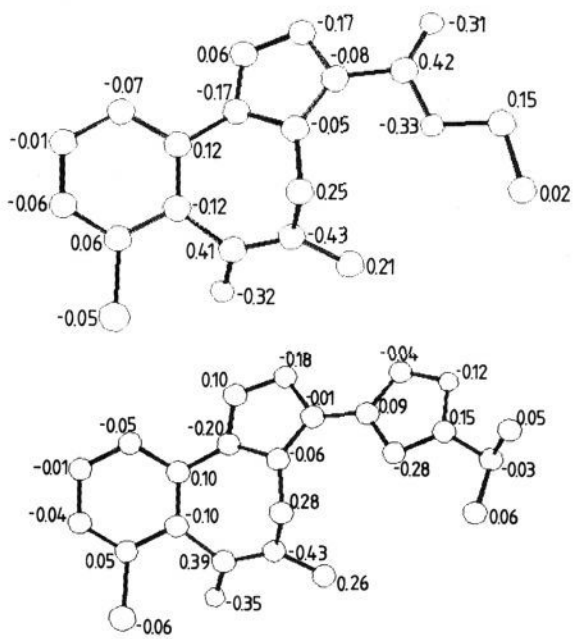


Figure 5. Partial charges for compounds **2b** (A) and **10h** (B) as determined by MOPAC.[22]

greater than that on the imidazole nitrogen (equivalent N-13; −0.31 versus −0.17), suggesting that the former will preferentially participate as the hydrogen-bond-acceptor

(20) Fryer, R. I.; Cook, C.; Gilman, N. W.; Walser, A. *Life Sci.* **1986**, *39*, 1947.
(21) QCPE 455 accessed via the CHEMQM software from Chemical Design Ltd.

site as predicted in the binding model for antagonists. The situation is reversed in oxadiazole **10h** (−0.18 charge on N-13 versus −0.04 on the oxadiazole nitrogen corresponding to the carbonyl oxygen), thereby supporting the proposal that a switch in acceptor site takes place in the oxadiazole partial agonists (Figure 4B).

In view of the preference for a 7-rather than 8-substituent observed in the binding data for **9–12**, it is necessary to modify another aspect of the proposed model. However, a rationalization of this finding is difficult to achieve since the effect of the 7-chloro substituent, while consistent throughout the oxadiazole series **9–12**, should also be carried over into the ester series **2**. The major structural difference between the 7- and 8-substituted series is the relative conformational mobility of the diazepine ring in each as revealed by variable-temperature NMR studies. The ^{1}H NMR spectra of compounds **9–12** (see the Experimental Section) were characterized by two well-separated signals in the range δ 4.2 to δ 5.9 arising from the diazepine ring methylene protons. The stereochemical assignment of these protons was made by comparison of their chemical shift with that of the proton at δ 5.0 in **19** whose configuration was known being derived from L-proline. Thus compounds **10a**, **10i**, **12d**, and **12p** achieved coalescence below 400 K whereas **10e** and **12l** were still at slow exchange at this temperature. Assuming that the bulky 7-substituent effectively locks the diazepine ring in a conformation recognized by the receptor, the entropic gain may well account for the increased binding affinity in this range of compounds.

Experimental Section

Chemical Methods. General Directions. Except where otherwise stated, the following procedures were adopted: all ^{1}H NMR spectra were recorded at 360 MHz on a Bruker AM 360 instrument fitted with a BVT 1000 variable-temperature unit (up to 410 K), mass spectra with a VG 70-250 mass spectrometer, and infrared spectra on a Perkin-Elmer 782 IR spectrometer. Organic solvents were purified when necessary by the methods described by D. D. Perrin, W. L. F. Armarego, and D. R. Perrin (*Purification of Laboratory Chemicals*; Pergamon: Oxford, 1966) or were purchased form the Aldrich Chemical Co. (Sureseal). Petroleum ether refers to that fraction having a boiling point range of 60–80 °C. All solutions were dried over sodium sulfate and evaporated on a Büchi rotary evaporator with a water bath temperature set to 40 °C or below. Thin-layer chromatography and preparative chromatography were carried out on silica using plates (Merck Art No. 5719) and gravity columns (Merck Art No. 7734) or LOBAR columns (Merck Art No. 10402), respectively. Melting points are uncorrected.

Method 1A. Ethyl 7-Chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylate (15) (X = 7-Cl). A solution of lithium diisopropylamide in THF (44 mL of a 1.5 M solution, 66 mmol) was added dropwise to a solution of ethyl isocyanoacetate (7.5 g, 66 mmol) in THF (100 mL) at −78 °C under nitrogen, and the resulting solution was stirred at −78 °C for 1 h. Meanwhile, NaH (2.3 g of a 55% dispersion in oil, 50 mmol) was added to a stirred solution of 6-chloro-3,4-dihydro-4-methyl-2*H*-1,4-benzodiazepine-2,5(1*H*)-dione (10 g, 44 mmol) in dimethylformamide (DMF) (100 mL) at room temperature. After 30 min, the DMF solution was cooled to −20 °C and diethyl chlorophosphate (7.2 mL, 50 mmol) added. The resulting solution was stirred for a further 30 min at −10 °C prior to the transfer via cannula to the solution of the isonitrile anion at −78 °C described above. After 2 h at −78 °C, the reaction was allowed to warm to −30 °C and acetic acid (3.6 g, 60 mmol) added. The residue obtained after evaporation of the solvent was dissolved in $CHCl_3$ (110 mL) and washed with water (100 mL) and again evaporated. Trituration of the residue with ether followed by recrystallization from CH_2Cl_2–Et_2O gave the required ester as a white solid (7 g; 47%): mp 224 °C (lit.[12] mp 229–230 °C); R_f 0.2 EtOAc, on silica; ^{1}H NMR ($CDCl_3$) δ 1.45 (3 H, t, J = 7 Hz, CH_2CH_3), 3.20 (3 H, s, NCH_3), 4.39–4.51 (2 H, m, CH_2CH_3), 4.40 (1 H, d, J = 16 Hz, equatorial CH), 5.16 (1 H, d, J = 16 Hz, axial CH), 7.33 (1 H, d, J = 6 Hz, H-8), 7.51 (1 H, t, J = 6 Hz, H-9), 7.59 (1 H, d, J = 6 Hz, H-10), 7.91 (1 H, s, H-1).

7-Chloro-5,6-dihydro-5-methyl-6-oxo-3-(3-propyl-1,2,4-oxadiazol-5-yl)-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (9h). Butyramide oxime (3 g, 29 mol) was added to a stirred suspension of powdered 4A molecular sieves (5 g) in anhydrous tetrahydrofuran (THF) (30 mL) under nitrogen. After the mixture was stirred at room temperature for 15 min, NaH (0.75 g of an 80% dispersion in oil, 28 mmol) was added in portions over 10 min. After the mixture was stirred for a further 40 min, a solution of the foregoing ester (4.6 g, 14 mmol) in THF (50 mL) was added and the resulting mixture heated at reflux for 1 h. The mixture was cooled to room temperature, acetic acid (1.68 g, 28 mmol) was added, and after stirring for 10 min, the mixture was filtered through Celite. The filtrate was diluted with dichloromethane (100 mL) and washed with water (100 mL) and then brine (100 mL). Evaporation of the solvent afforded a pale yellow solid which was purified by chromatography on silica by eluting with EtOAc. Recrystallization of the product from CH_2Cl_2–Et_2O afforded the title compound as white crystals (4.5 g; 89%): mp 173 °C; R_f 0.3 EtOAc on silica; MS m/z 358 (M^+ + 1), 291 (base peak), 273, 239, 115; ^{1}H NMR ($CDCl_3$) δ 1.03 (3 H, t, J = 7 Hz, CH_2CH_3), 1.84–1.89 (2 H, m, CH_2CH_3), 2.79 (2 H, t, J = 7 Hz, CH_2CH_2), 3.21 (3 H, s, NMe), 4.51 (1 H, d, J = 16 Hz, equatorial CH), 5.2 (1 H, d, J = 16 Hz, axial CH), 7.37 (1 H, d, J = 7 Hz, H-8), 7.54 (1 H, overlapping dd, each J = 7 Hz, H-9), 7.61 (1 H, d, J = 7 Hz, H-10) and 8.03 (1 H, s, H-1). Anal. ($C_{17}H_{16}ClN_5O_2$) C, H, N.

Method 1B. 7-Chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic Acid (20). Ester 15 (X = 7-Cl) (18.3 g, 57.2 mmol) in methanol (180 mL) was treated with 2 N NaOH (975 mL, 150 mmol) over 1 h. The methanol was evaporated and the resulting aqueous solution cooled to 5 °C and acidified to pH 2 with concentrated hydrochloric acid. After 30 min at 0 °C, the precipitated solid was collected by filtration, washed with water and then acetone, and dried over P_2O_5 to give the acid (14.0 g, 84%): mp 262 °C; MS m/z 290 (M^+ − 1); ^{1}H NMR (d_6-DMSO) δ 3.02 (3 H, s, CH_3), 4.49 (1 H, d, J = 16 Hz, equatorial CH), 4.98 (1 H, d, J = 16 Hz, axial CH), 7.68 (3 H, s, C_6H_3) and 8.39 (1 H, s, H-1). Anal. ($C_{13}H_{10}ClN_3O_3$) C, H, N.

7-Chloro-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carbonitrile (21). Acid **20** (16.7 g, 57.5 mmol) in CH_2Cl_2 (250 mL) was treated at 15 °C first with Et_3N (8.8 mL, 63.2 mmol) and then with ethyl chloroformate (6.0 mL, 63 mmol). After 1 h at 15–20 °C, dry ammonia gas was bubbled through the reaction mixture until no further precipitation occurred (about 30 min). The reaction mixture was poured into water and the material isolated from the organic layer (14.0 g) used directly as follows. The rigorously dried material (14.0 g, 40 mmol) from above was suspended in dry dioxane (250 mL) containing redistilled pyridine (100 mL) and cooled to 0 °C and trifluoroacetic anhydride (14.5 mL, 101 mmol) was added. After 16 h at 20 °C, the mixture was diluted with CH_2Cl_2 (1 L) and washed with water (4 × 250 mL). The organic layer was treated with activated charcoal and filtered and the filtrate was evaporated to a small volume. Addition of Et_2O followed by isolation of the precipitated solid yielded the desired nitrile (11.1 g, 70%): mp 139–140 °C; IR ν_{max} (CH_2Cl_2 solution) 2240 and 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.22 (3 H, s, NCH_3), 4.38 (1 H, d, J = 16.5 Hz, equatorial CH), 4.51 (1 H, d, J = 16.5 Hz, axial CH), 7.32 (1 H, d, J = 7.9 Hz, H-8), 7.55 (1 H, overlapping dd, J = 6.4 and 7.9 Hz, H-9), 7.62 (1 H, d, J = 6.4 Hz, H-10), and 7.94 (1 H, s, H-1). Anal. ($C_{13}H_9ClN_4O$) C, H, N.

7-Chloro-5,6-dihydro-3-(5-isopropyl-1,2,4-oxadiazol-3-yl)-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (10h). To a solution of nitrile **21** (11.88 g, 43 mmol) in EtOH (300 mL) and H_2O (20 mL) were added hydroxylamine hydrochloride (4.3 g, 61 mmol) and K_2CO_3 (8.5 g, 61 mmol), and the mixture was heated under reflux for 1 h. The solvents were removed in vacuo, and the dried residue was dissolved in isobutyric anhydride (65 mL) heated at 160 °C for 4.5 h. After cooling, water (60 mL) was added to the mixture and this extracted with dichloromethane. The material isolated from the organic extracts was triturated with petroleum ether (bp 40–60 °C) and the remaining residue dissolved in dichloromethane and treated with

activated charcoal. Evaporation of the solvent followed by recrystallization from EtOAc gave the desired oxadiazole (6.83 g, 44%): mp 165 °C; MS m/z 3.58 ($M^+ + 1$), 324, 273, 239, and 184; ^1H NMR ($CDCl_3$) δ 1.48 (6 H, d, J = 7 Hz, 2 CH_3), 3.21 (3 H, s, NCH_3), 3.28–3.33 (1 H, m, $CH(CH_3)_2$), 4.47 (1 H, d, J = 15.9 Hz, equatorial CH), 5.19 (1 H, d, J = 15.9 Hz, axial CH), 7.34 (1 H, d, J = 7.7 Hz, H-8), 7.49–7.59 (1 H, overlapping dd, J = 7.7 and 8.0 Hz, H-9), 7.57 (1 H, d, J = 8.0 Hz, H-10), and 8.01 (1 H, s, H-1). Anal. ($C_{17}H_{16}ClN_5O_3$) C, H, N.

Method 1C. 3-[(*N*-Formylamino)methyl]-5-isopropyl-1,2,4-oxadiazole. (a) *N*-Formylaminoacetonitrile. Aminoacetonitrile hydrochloride (100 g, 1.08 mol) suspended in trimethyl orthoformate (500 mL, 4.6 mol) was heated under reflux for 0.5 h. After cooling, the mixture was filtered rapidly through a cotton wool plug and then evaporated. The residue was fractionally distilled under reduced pressure to give *N*-formylaminoacetonitrile (52.9 g, 58%), bp 130 °C (0.3 mmHg).

(b) *N*-Formylaminoacetamide Oxime. Hydroxylamine hydrochloride (104.2 g, 1.5 mol) dissolved in boiling CH_3OH (460 mL) was treated with a solution of $NaOCH_3$ (77.22 g, 1.43 mol) in CH_3OH (450 mL). After 10 min at reflux temperature, the mixture was cooled and NaCl removed by filtration. The solution was then added at 0 °C to *N*-formylaminoacetonitrile (114.6 g, 1.36 mol) and the mixture stirred in the dark for 16 h at 5 °C. After cooling to −10 °C, the required amide oxime was filtered off, washed with cold CH_3OH, and dried (117 g, 67%).

(c) 3-[(*N*-Formylamino)methyl]-5-isopropyl-1,2,4-oxadiazole. To a solution of NaOEt (from 4.6 g of Na dissolved in 1 L of EtOH, 0.2 mol) under nitrogen were added crushed molecular sieves (100 g) and the *N*-formylaminomethyl acetamide oxime (100 g, 0.85 mol) followed by ethyl isobutyrate (205 g, 1.77 mol). The resulting mixture was heated under reflux for 6 h, cooled, and filtered through hyflo. The oil obtained from the filtrate after evaporation of the EtOH was taken into CH_2Cl_2 and treated with activated carbon. Reisolation gave an orange oil (92 g, 64%), which was used without purification in the next step: ^1H NMR ($CDCl_3$) δ 1.28 (6 H, d, J = 7 Hz, 2 CH_3), 3.12 (1 H, septet, J = 7 Hz, CH), 4.74 (2 H, d, J = 5.8 Hz, CH_2), 6.88–6.98 (1 H, m, NH), 8.19 (1 H, s, CHO).

3-(Isocyanomethyl)-5-isopropyl-1,2,4-oxadiazole (18) (R = $CH(CH_3)_2$). The foregoing [(formylamino)methyl]oxadiazole (120 g, 0.71 mol) and Et_3N (445 mL, 3.2 mol) were stirred mechanically in dry CH_2Cl_2 (800 mL) under nitrogen at −25 °C. Phosphorus oxychloride (66 mL, 0.71 mol) was added dropwise over 50 min while the temperatur was kept below −5 °C. After 1 h, a solution of Na_2CO_3 (113.4 g, 1.07 mol) in water (600 mL) was added dropwise at 10 °C and stirring continued for a further 1 h without cooling. The mixture was filtered through hyflo and evaporation of the organic layer afforded the required isonitrile (90 g): bp 100 °C (0.15 mmHg); ^1H NMR ($CDCl_3$) δ 1.37 (6 H, d, J = 6.9 Hz, $CH(CH_3)_2$), 3.21 (1 H, septet, J = 6.9 Hz, CH), and 4.69 (2 H, s, CH_2).

7-Chloro-5,6-dihydro-3-(5-isopropyl-1,2,4-oxadiazol-3-yl)-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (10h). Dione 13 (X = 6-Cl) (22.45 g, 100 mmol) in dry DMF (200 mL) stirred under nitrogen at 25 °C was treated portionwise with NaH (3.15 g of an 80% dispersion in oil, 105 mmol). After 1 h the mixture was cooled to −30 °C and diethyl chlorophosphate (18.1 g, 105 mmol) was added over 15 min. This mixture was stirred at about −20 °C for 1.5 h when a clear solution was obtained. Meanwhile lithium diisopropylamide (80 mL of a 1.5 M solution in cyclohexane, 120 mmol) was added over 20 min to a solution of 3-(isocyanomethyl)-5-isopropyl-1,2,4-oxadiazole (18.1 g, 120 mmol) in THF (200 mL) stirred under nitrogen at −70 °C. After 1.5 h at −70 °C the iminophosphate solution from above was added over 0.5 h maintained at a temperature of −70 °C. After a further 2 h at this temperature, the reaction mixture was allowed to warm to 0 °C over 0.5 h and HOAc added to adjust the pH of the solution to 7. The residue, after evaporation of the solvents, was partitioned between CH_2Cl_2 and water and the material was isolated from the organic layer and was purified by chromatography on silica with EtOAc. The crude product (12.1 g) was twice recrystallized from EtOAc to give the required oxadiazole (4.9 g, 14%), mp 165 °C, having physical data identical with the previous sample.

Method 2A. 7-Chloro-3-(3-isopropyl-1,2,4-oxadiazol-5-yl)-6-phenyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (11p). Isobutyramide oxime (0.765 g, 7.5 mmol) was added to a suspension of powdered 4A molecular sieves (3 g) in anhydrous THF (50 mL) under nitrogen. After the mixture was stirred at room temperature for 15 min, NaH (0.327 g of 85% dispersion in oil, 7.5 mmol) was added and the mixture stirred for a further 45 min. A solution of the ethyl 7-chloro-6-phenyl-4*H*-imidazo[1,5-*a*]-[1,4]benzodiazepine-3-carboxylate (1.83 g, 5 mmol) in THF (30 mL) was then added and the resulting mixture heated at reflux for 2 h. The reaction mixture was then cooled to room temperature and acetic acid (0.045 g, 7.5 mmol) added. After stirring at room temperature for 20 min, the mixture was filtered through Celite and the filtrate diluted with CH_2Cl_2 (100 mL). The solution was then washed with water (100 mL) and then brine (100 mL), and the residue obtained after evaporation of the CH_2Cl_2 was purified by chromatography on silica by eluting with EtOAC. Recrystallization (CH_2Cl_2/Et_2O) of the combined pure fractions afforded the req d oxadiazole (0.90 g): mp 251–252 °C; R_f 0.5 in EtOAc on silica; MS m/z 403 (M^+), 374, 317, 291, 188; ^1H NMR ($CDCl_3$) δ 1.41 (6 H, d, J = 7 Hz, 2 CH_3), 3.14–3.22 (1 H, m, $CH(CH_3)_2$), 4.22 (1 H, d, J = 16 Hz, equatorial C*H*), 6.07 (1 H, d, J = 16 Hz, axial CH), 7.2–7.6 (8 H, m, Ar), 8.09 (1 H, s, H-1). Anal. ($C_{22}H_{18}N_5OCl\cdot 0.1\cdot H_2O$) C, H, N.

Method 2B. 6-Phenyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carboxylic Acid. Ethyl 6-phenyl-4*H*-imidazo-[1,5-*a*][1,4]benzodiazepine-3-carboxylate (5.96 g, 18 mmol) in ethanol (27 mL) was treated at room temperature with 2 N NaOH (27 mL, 54 mmol). The solution was then heated under reflux for 0.5 h and cooled to 0 °C and 2 N HCl (27 mL) added. The solid that precipitated was collected by filtration, washed with cold ethanol and then ether, and dried over P_2O_5 to afford the acid (5.21 g, 95%), mp 234–236 °C.

6-Phenyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine-3-carbonitrile (22). The acid (5.45 g, 18 mmol) in CH_2Cl_2 (100 mL) containing Et_3N (1.91 g, 18.9 mmol) was treated at 15 °C with ethyl chloroformate (2.05 g, 18.9 mmol) under dry nitrogen. After 1 h at 15–20 °C, dry NH_3 gas was bubbled through the reaction mixture until no further precipitation was noticed (about 0.5 h). The mixture was poured into water and the aqueous layer washed with fresh CH_2Cl_2 (three times). The combined organic extracts afforded the intermediate amide as a white solid (5.58 g) which was used without further purification. All of this material (18.3 mmol) suspended in dioxane (250 mL) and pyridine (11.6 g, 146 mmol) was treated at 10 °C with trifluoroacetic anhydride (15.4 g, 73 mmol) and then left at 20 °C for 5 h. The mixture was evaporated and the residue partitioned between CH_2Cl_2 and water. The material isolated from the organic extracts was purified by chromatography on silica eluting with hexane/EtOAc (1:1) to give the required nitrile (3.3 g): mp 154 °C; MS m/z 284 (M^+), 127, 110, and 77; ^1H NMR ($CDCl_3$) δ 4.16 (1 H, d, J = 10 Hz, equatorial CH), 5.40 (1 H, d, J = 10 Hz, axial CH), 7.26–7.73 (9 H, m, C_6H_4 and C_6H_5), and 7.96 (1 H, s, H-1). Anal. ($C_{18}H_{12}N_4$) C, H, N.

3-(5-Ethyl-1,2,4-oxadiazol-3-yl)-6-phenyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (12b). The foregoing nitrile (3.0 g, 10.6 mmol) in EtOH (200 mL) and water (10 mL) was treated with K_2CO_3 (2.19 g, 15.9 mmol) and then hydroxylamine hydrochloride (1.11 g, 15.9 mmol) at reflux for 5 h. The mixture was evaporated and the residue triturated with ice-cold water (20 mL) and the solid collected by filtration and dried over P_2O_5 to give the intermediate crude amide oxime (2.91 g). Part of this material (950 mg) in propionic anhydride (10 mL) was heated at 140 °C under nitrogen for 5 h. The cooled mixture was triturated with EtOAc at −10 °C and the resulting crude product purified by chromatography on silica in EtOAC/hexane (1:1). Recrystallization from EtOAc gave the desired oxadiazole (380 mg): mp 184–185 °C; MS m/z 355 (M^+), 284 (base peak), 158, 98, and 69; ^1H NMR ($CDCl_3$) δ 1.45 (3 H, t, J = 5.8 Hz, CH_3), 2.97 (2 H, q, J = 5.8 Hz, CH_2CH_3), 4.17 (1 H, br d, J = 10.2 Hz, equatorial CH), 6.08 (1 H, br d, J = 10.2 Hz, axial CH), 7.26–7.68 (9 H, m, C_6H_4 and C_6H_5), and 8.05 (1 H, s, H-1). Anal. ($C_{21}H_{17}N_5O\cdot 0.2H_2O$) C, H, N.

Method 2C. 7-Fluoro-3-(5-methyl-1,2,4-oxadiazol-3-yl)-6-phenyl-4*H*-imidazo[1,5-*a*][1,4]benzodiazepine (12t). Lithium diisopropylamide (11.8 mL of a 1.5 M solution in cyclohexane, 17.7 mmol) was added to 3-(isocyanomethyl)-5-methyl-1,2,4-oxadiazole (2.18 g, 17.7 mmol) in THF (100 mL) at −78 °C and the

mixture stirred for 1.5 h at this temperature. Meanwhile NaH (0.45 g of an 80% dispersion in oil, 14.2 mmol) was added to 1,3-dihydro-6-fluoro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (3.0 g, 11.8 mmol) in DMF (100 mL) and the mixture stirred at 20 °C for 30 min before cooling to −40 °C. Diethyl chlorophosphate (2.05 mL, 14.2 mmol) was added and the mixture allowed to stir at −10 °C for 1 h and then cooled to −78 °C. The DMF solution was then added to the solution of the isonitrile anion described above at −78 °C and stirring continued for 3 h. Acetic acid (2 mL) was then added, the solvents were evaporated, and the residue in CH_2Cl_2 (250 mL) was washed with water (2 × 20 mL). Evaporation of the CH_2Cl_2 followed by chromatography of the residue on silica with EtOAc gave the desired oxadiazole as a white solid (0.97 g, 15%): mp 215–216 °C; R_f 0.40 on silica in EtOAc; MS m/z 359 (M^+), 302 (base peak), 275, 249, 199, 172, 104; ^1H NMR ($CDCl_3$) δ 2.62 (3 H, s, CH_3), 4.24 (1 H, d, J = 12.6 Hz, equatorial CH), 6.14 (1 H, d, J = 12.6 Hz, axial CH), 7.19–7.72 (8 H, m, C_6H_3 and C_6H_5), 8.06 (1 H, s, H-1). Anal. ($C_{20}H_{14}FN_5O$) C, H, N.

Biochemical Methods. (a) Brain Membrane Preparation. Cerebral cortices of Sprague–Dawley (250–300 g) rats were dissected and homogenized in 9 vol (weight/volume) of ice-cold 0.32 M sucrose by 10 stokes in a glass Teflon homogenizer at 500 rpm. All further procedures were carried out at 4 °C. Homogenate were centrifuged at 1000*g* for 10 min and the supernatant was recentrifuged at 10000*g* for 20 min. The upper "buffy coat" layer of the pellet was suspended in 20 vol of ice-cold water and centrifuged at 50000*g* for 20 min. The water washing step was repeated three times and the resultant pellet stored at −20 °C for at least 18 h.

(b) [^{3}H]-Ro 15-1788 Binding Assay. On the day of the experiment, the cortical membranes were thawed and mixed with 30 vol of a modified Krebs buffer (in mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 5, HEPES 20, KH_2PO_4 1.2, $CaCl_2$ 2.5, and D-glucose 11; pH 7.4) and left at room temperature (23 °C) for 30 min and then centrifuged at 50000*g* for 10 min. The pellet was resuspended in 30 vol of Krebs/HEPES buffer and incubated at room temperature for 15 min before centrifugation. This washing step was repeated three more times. For the determination of [^{3}H]-Ro 15-1788 binding, the washed membranes were homogenized in 0.75 mL of Krebs/HEPES buffer per sample, each containing 0.2–0.5 mg of protein and added to polycarbonate test tubes containing 100 μL of 5 nM [^{3}H]-Ro 15-1788, 100 μL of displacer or buffer (for determination of total binding), and 50 μL of buffer or of 6 mM GABA solution (for the determination of GABA shift). To control for possible uptake of GABA, nipecotic acid (10^{-4} M) was included in the incubation medium in all experiments. Nonspecific binding was defined by 3 μM clonazepam. Samples were incubated at 30 °C for 60 min and terminated by rapid filtration through Whatman GF/B filters in a Brandel M24-R cell harvester followed by 2 × 4 mL washes of ice-cold 0.9% NaCl solution. The filters were soaked in 10 mL of Hydrofluor overnight, and radioactivity was determined by liquid scintillation counting at 41% counting efficiency. Potencies for displacement (IC_{50}) were determined from data obtained with at least four concentrations of the displacer by computer-assisted iterative curve fitting.

X-ray Crystallography. X-ray Crystal Structure Analysis of 10h-I. Suitable crystals of **10h** ($C_{17}H_{16}N_5O_2Cl$) for X-ray diffraction studies formed from ethyl acetate with space group symmetry of *Pbca* and cell constants of a = 7.203 (1) Å, b = 21.464 (1) Å, and c = 21.776 (2) Å for Z = 8 and a calculated density of 1.412 g/cm^3. Of the 2294 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 1895 were observed ($I > 3\sigma(I)$) for $2\theta_{max}$ = 115°. The structure was solved with a direct methods approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[22] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\sum\omega(|F|_o - |F_c|)^2$ with $\omega = 1/(\sigma F_o)^2$ was minimized; R = 0.056; WR = 0.080; S = 3.03; $(\Delta/\sigma)_{max}$ = 2.0; $(\Delta e)_{max}$ = 0.41/e A^{-3}; where R is the unweighted residual, WR is the weighted residual, S is the standard deviation of an observation of unit weight, $(\Delta/\sigma)_{max}$ is the maximum shift/error of the final refinement cycle, and $(\Delta e)_{max}$ is the maximum residual peak in the final difference Fourier synthesis. No abnormally short intermolecular contacts were noted. Tables I, II, and III containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material. Figure 2A is a computer-generated perspective drawing of **10h-I** from the final X-ray coordinates.

X-ray Crystal Structure Analysis of 10h-II. Suitable crystals of **10h** ($C_{17}H_{16}N_5O_2Cl$) for X-ray diffraction studies formed from acetone with space group symmetry of $P\bar{1}$ and cell constants of a = 7.944 (1) Å, b = 13.685 (1) Å, c = 17.858 (2) Å, α = 104.53 (1)°, β = 93.29 (1)°, and γ = 88.00 (1)° for Z = 4 and a calculated density of 1.370 g/cm^3. A single molecule of acetone was found in the asymmetric unit. Of the 5140 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 4214 were observed ($I > 3\sigma(I)$) for $2\theta_{max}$ = 115°. The structure was solved with a direct methods approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[22] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\sum\omega(|F_o| - |F_c|)^2$ with $\omega = 1/(\sigma F_o)^2$ was minimized; R = 0.052; WR = 0.074; S = 2.83; $(\Delta/\sigma)_{max}$ = 4.1; $(\Delta e)_{max}$ = 0.39 e A^{-3}. No abnormally short intermolecular contacts were noted. Tables IV, V, and VI containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material.

X-ray Crystal Structure Analysis of 12o. Suitable crystals of **12o** ($C_{22}H_{18}N_5OCl$) for X-ray diffraction studies formed from ethyl acetate with space group symmetry of $C2/c$ and cell constants of a = 42.164 (9) Å, b = 7.009 (2) Å, c = 13.267 (2) Å, and β = 92.57 (1)° for Z = 8 and a calculated density of 1.370 g/cm^3. Of the 2639 reflections measured with an automatic four circle diffractometer equipped with Cu radiation, 2180 were observed ($I > 3\sigma(I)$) for $2\theta_{max}$ = 114°. The structure was solved with a direct methods approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[22] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\sum\omega(|F_o| - |F_c|)^2$ with $\omega = 1/(\sigma F_o)^2$ was minimized; R = 0.050; WR = 0.066; S = 2.47 $(\Delta/\sigma)_{max}$ = 1.2; $(\Delta e)_{max}$ = 0.39 e A^{-3}. No abnormally short intermolecular contacts were noted. Tables VII, VIII, and IX containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material. Figure 2B is a computer-generated perspective drawing of **12o** from the final X-ray coordinates.

Acknowledgment. We thank our colleagues A. Carpenter, A. Christensen, and P. Carter for their contributions to this work.

Supplementary Material Available: Tables of the atomic positional and thermal parameters, bond distances, and bond angles for **10h** and **12o** (12 pages). Ordering information is given on any current masthead page.

(22) The following library of crystallographic programs was used: Sheldrick, G. M. SHELXS-86, University of Gottingen, Gottingen, West Germany, 1986. Johnson, C. K. ORTEP-II; Oak Ridge National Laboratory, Oak Ridge, TN, 1970. Okaya, Y.; et al SDP Plus V1.1, B.A. Frenz and Associates, Colledge Station, TX, 1984.