1-Azacycloalkyl-1,4-benzodiazepin-2-ones with Antianxiety–Antidepressant Actions

Tsutomu Sugasawa,*† Makoto Adachi,† Kazuyuki Sasakura,† Akira Matsushita,*‡ Masami Eigyo,‡ Teruo Shiomi,‡ Haruyuki Shintaku,‡ Yukio Takahara,‡ and Shunji Murata‡

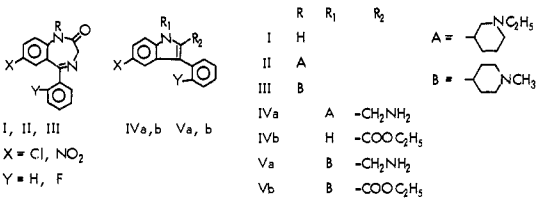
Divisions of Organic Chemistry and Neuropharmacology, Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553, Japan. *Received October 4, 1984*

A series of 1-azacycloalkyl-1,4-benzodiazepin-2-ones were synthesized from 1-azacycloalkyl-2-benzoylanilines **2–4** and corresponding imines **5** and **6** and then evaluated for their central nervous system activities. Pharmacological data showed that some of these compounds have potent antidepressant properties, as assessed by their antagonism of tetrabenzine (TBZ) induced ptosis and their inhibition of [^{3}H]norepinephrine uptake into rat brain synaptosomes, as well as their moderate antianxiety properties of preventing of pentylenetetrazol (PTZ) convulsion, suppressing conflict behavior, and displacing potential for [^{3}H]diazepam binding. Introduction of a halogen substituent at position 7 of the 1,4-benzodiazepine ring lengthened the anti-PTZ effects, although the peak effect was slightly reduced and clearly enhanced the anti-PTZ and anticonflict properties. Introduction of Cl to the ortho position of the phenyl ring at position 5 greatly reduced the antidepressant properties. The secondary amine function of the azacyclic ring at position 1 was essential for the production of the antidepressant properties. Of these new series, 7-fluoro-5-(2-fluorophenyl)-1,3-dihydro-1-(4-piperidinyl)-2*H*-1,4-benzodiazepin-2-one (**10d**) has the potential to become a useful antidepressant drug with a moderate antianxiety property.

As the chemistry, psychopharmacology, and structure–activity relationships of 1,4-benzodiazepines (**1**)[1] have been clearly established, the possibility for finding a new type of **1**, which is readily accessible by simple modification and yet retains favorable psychopharmacological properties, is small. However, our exclusive ortho acylation reaction of anilines using boron trichloride and nitriles[2] made possible the one-step synthesis of 2-acyl-*N*-azacycloalkylanilines **2–4** and the corresponding imines **5** and **6**, and we synthesized an almost inaccessible[3] series of 1,4-benzodiazepines (**7–12**) having a piperidine or pyrrolidine moiety directly attached at position 1 (Scheme I). Although detailed investigation of the structure–activity relationships of **1** revealed that substitution of basic aminoalkyl side chains at position 1 generally weakens anticonvulsant activity,[4] we found that some of our new 1,4-benzodiazepines possess the pharmacological features of potent antidepressants together with those of anxiolytics. The combined antianxiety–antidepressant actions of benzodiazepine derivatives have been reported in recent years.[5]

Chemistry. With use of **2** and **3** (R_3 = alkyl) as starting material, **7** and **8** were synthesized by the conventional ways[1] shown in Scheme II. Namely, **2** and **3** (R_3 = alkyl) were treated with chloroacetyl chloride to give **13** and **14**, which were cyclized with ammonium carbonate instead of ammonia in order to avoid the formation of **15** by Smiles' rearrangement (method A, Scheme II). However, **13** (R_1 = CH_3, X = 4-NO_2, Y = 2-Cl) gave **15** (X = 4-NO_2, Y = 2-Cl) as the main product (25%), so the desired **7n** (Table I) was synthesized by nitration of **7b**. Alternatively, **2** and **4** (R_3 = CH_3) were condensed with phthaloylacetyl chloride to give **16** and **17**, which were subjected to hydrazinolysis (method B). This method generally gave **15** as a byproduct; **16** (X = 9-Cl, Y = 2-F) gave **15** (X = 9-Cl, Y = 2-F) in 50% yield besides the target compound **7s** (14%). Compounds **7** and **9** were obtained more conveniently, when the 2-acylanilinimines **5** and **6** were condensed with glycine ethyl ester hydrochloride to give imino esters **18** and **19**, which were cyclized under acidic conditions in which polyphosphoric acid (PPA) was found to be most favorable. Compound **10** could also be obtained directly from **5** (R_3 = H) (method C).

To synthesize 1-piperidinyl-1,4-benzodiazepin-2-ones **10** and **11**, **2** and **3** (R_3 = CH_3) were converted into (benzyloxycarbonyl)piperidinyl derivatives **24** and **25** by successive treatment with ethyl chloroformate, 6 N hydrochloric acid, and benzyl chloroformate via compounds **20** and **21** and compounds **22** and **23**. Method A and B were used to cyclize **24** and **25** to the corresponding 1,4-benzodiazepin-2-ones **26** and **27**. Decarbobenzoxylation was conducted by treatment with a solution of aluminum trichloride and anisole in dichloromethane and nitromethane[6] or by hydrolysis with trifluoroacetic acid (me-



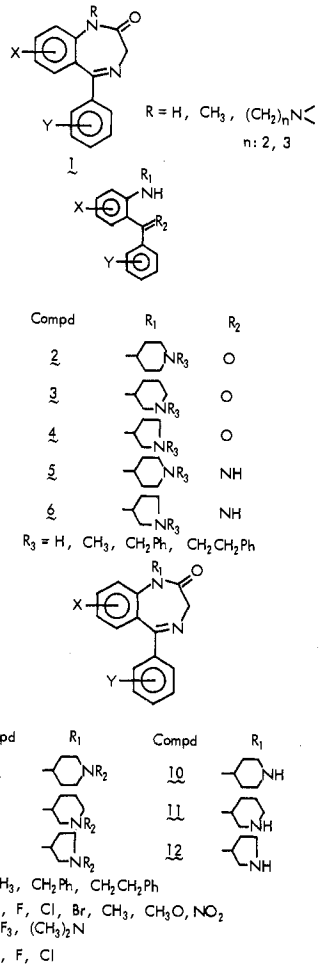
work, we confirmed that treatment of I (X = Y = Cl) with 4-chloro-*N*-methylpiperidine (VI) in the presence of NaH in toluene and DMF (1:1) under reflux for 4 h gave no trace of III (X = Y = Cl), with I being recovered in quantitative yield. Also, treatment of IVb (X = Cl, Y = H) with VI under similar conditions or by transfer catalysis (benzyldimethylcetylammonium chloride) did not give the desired piperidinylindole Vb (X = Cl, Y = H), with the starting material being recovered in almost quantitative yield.

(1) (a) Sternbach, L. S. "The Benzodiazepines"; Raven Press: New York, 1973; pp 1–26. (b) Sternbach, L. S. *J. Med. Chem.* **1979**, *22*, 1.
(2) Adachi, M.; Sasakura, K.; Sugasawa, T. *Chem. Pharm. Bull.*, in press.
(3) After the completion of our work, we were informed about a patent application from Sumitomo Chemical Co., Ltd., Osaka, Japan, in 1968 (Ger. Offen. 1 929 810, 1970; *Chem. Abstr.* **1970**, *72*, 100773j), which partly anticipated our work. It was stated that compound II could be obtained by treatment of I with 1-ethyl-3-chloropiperidine in the presence of NaH in toluene and DMF in an unspecified yield. Alternatively, II could be obtained by oxidative ring closure of IVa in an unspecified yield. But no experimental data was given on the synthesis of IVa, the starting material, or compounds III and Va. (Compound III was supposed to have been obtained from I or Va in a reaction similar to that for obtaining II.) During our
(4) Sternbach, L. H.; Archer, G. A.; Earley, J. V.; Fryer, R. I.; Reeder, E.; Wasyliw, N.; Randall, L. O.; Banziger, R. *J. Med. Chem.* **1965**, *8*, 815.
(5) Kaiser, C.; Bondinell, W. E. *Annu. Rep. Med. Chem.* **1981**, *16*, 4. Bondinell, W. E.; Kaiser, C. *Ibid.* **1982**, *17*, 43. Ohnmacht, C. J.; Malick, J. B.; Frazee, W. J. *Ibid.* **1983**, *18*, 42.
(6) Tsuji, T.; Kataoka, T.; Yoshioka, M.; Sendo, Y.; Nishitani, Y.; Hirai, S.; Maeda, T.; Nagata, W.; *Tetrahedron Lett.* **1979**, 2793.

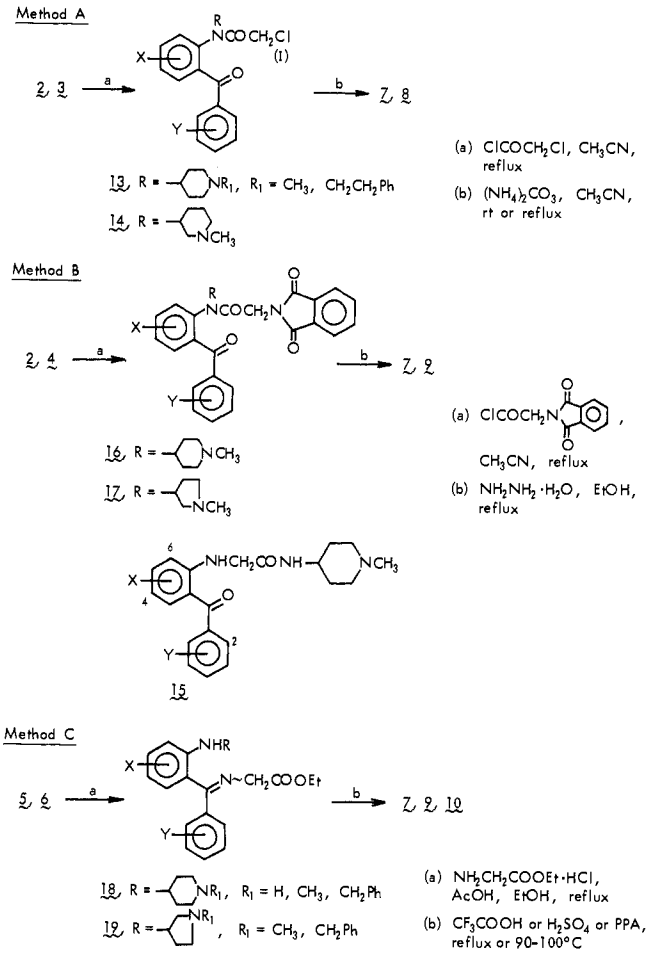
† Division of Organic Chemistry.
‡ Division of Neuropharmacology.

0022-2623/85/1828-0699$01.50/0

Scheme I



Scheme II



thod D, Scheme III). In a more facile reaction, **10** and **12** could be obtained via [*N*-(ethoxycarbonyl)piperidinyl]- and [*N*-(ethoxycarbonyl)pyrrolidinyl]-1,4-benzodiazepin-2-ones **28** and **29** produced by dealkylative ethoxycarbonylation of **7** ($R_2 = CH_3$) and **9** ($R_2 = CH_2Ph$) followed by mild hydrolysis with use of dialkyl sulfide and methanesulfonic acid.[7] However, in the present study, we adopted an even more convenient method in which demethylation was conducted with α-chloroethyl chloroformate in a one-pot operation[8] (method E). Compound **28** could also be obtained from **20** by method A or B. Similarly, *N*-acetyl derivative **30** was synthesized from **31**, which had been prepared by acetylation of **22**.

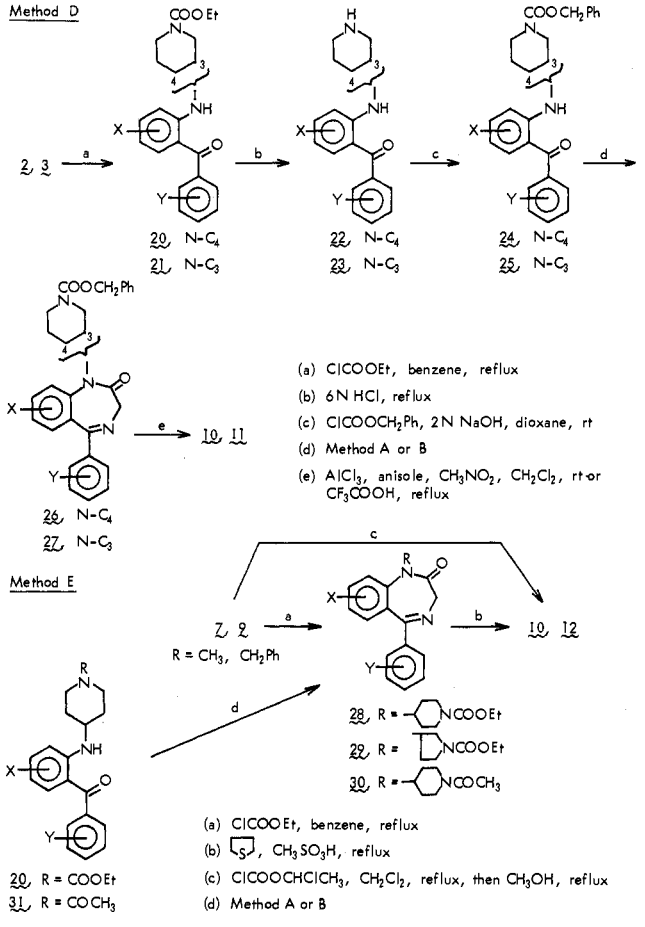
3-Hydroxy derivatives **34** were produced by successive treatment of the *N*-benzyloxycarbonyl derivative **26** with 30% hydrogen peroxide and acetic anhydride followed by simultaneous deprotection with aluminum trichloride and anisole as described in method D via **32** and **33** (method F, Scheme IV).

Pharmacology. The antidepressant and anxiolytic activities of 1-azacycloalkyl-1,4-benzodiazepin-2-ones were primarily assessed by their prevention of tetrabenazine (TBZ) induced ptosis and pentylenetetrazol (PTZ) intoxication in mice, respectively. The antianxiety activities of selected compounds were followed up by observing anticonflict effect in rats. Also examined with selected compounds were the uptake inhibition of [^{3}H]norepinephrine (NE) into rat brain synaptosomes[9] and the displacing potential for [^{3}H]diazepam (DZ) binding in rat brain.[10] Pharmacological data are summarized in Tables III–V.

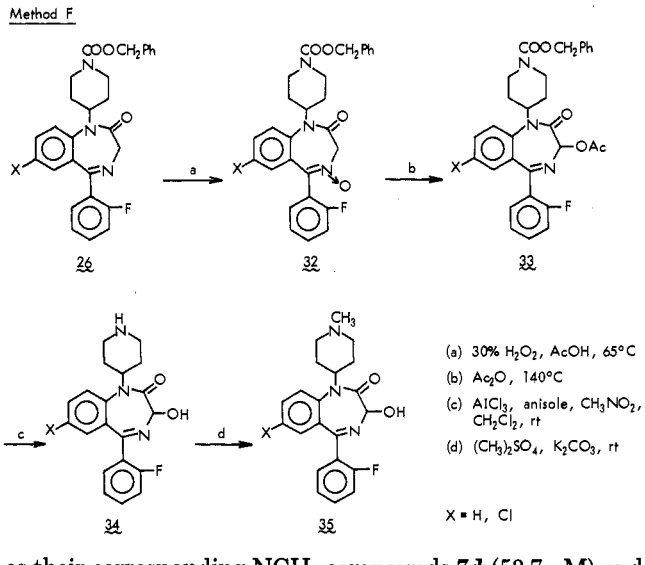
Most of the test compounds antagonized TBZ-induced ptosis in mice, when administered 1 h prior to TBZ. The potency was generally higher in compounds with the secondary amine function on the piperidine or pyrrolidine ring in position 1 of 1,4-benzodiazepine (NH) than their corresponding *N*-methyl congeners (NCH_3). This rule applied to six pairs of compounds: **7d–10d**, **7e–10e**, **7g–10g**, **7h–10h**, **7i–10i**, and **9a–12a**. However, no large difference was seen in their potencies when the test was carried out 4 h after administration. For example, the respective ED_{50} values of **7i** and **10i** with the pretreatment schedule of 1 h were 2.88 and 0.76 mg/kg, while the values with the 4-h pretreatment schedule were nearly equal, 0.69 and 0.64 mg/kg, po. Upon oral administration, the most active compound was **10a**, with equivalent activity shown by its NCH_3 compound (**7a**). However, **10a** was about 4 times as potent as **7a** when administered subcutaneously. This result indicates that the antidepressant profile judged from the anti-TBZ effect may be intrinsic for the NH compounds and that biotransformation, i.e., demethylation, is required for the NCH_3 compounds to elicit this action. Biochemical data on the uptake inhibition of [^{3}H]NE support this assumption. The NH compounds **10d** and **10h** prevented the uptake of NE with the respective IC_{50} values of 0.08 and 0.56 μM, being 70–700 times as potent

(7) Irie, H.; Nakanishi, H.; Fujii, N.; Mizuno, Y.; Fushimi, T.; Funakoshi, S.; Yajima, H. *Chem. Lett.* **1980**, 705.
(8) Olofson, R. A.; Martz, J.; Senet, J. P.; Piteau, M.; Malfroot, T. *J. Org. Chem.* **1984**, *49*, 2081.
(9) Randrup, A.; Braestrup, C. *Psychopharmacology* **1977**, *53*, 309.
(10) Möhler, H.; Okada, H. *Life Sci.* **1977**, *20*, 2101.

Scheme III



Scheme IV



as their corresponding NCH_3 compounds **7d** (53.7 μM) and **7h** (39.9 μM). Imipramine antagonized TBZ-induced ptosis with an ED_{50} value of 2.14 mg/kg, po and its IC_{50} value for NE-uptake inhibition was 0.09 μM. Thus, **7a** and **10a** were more active in the present tests than imipramine.

The introduction of substituents to the benzodiazepine moiety and/or the phenyl ring at position 5 gave rise to interesting changes in both antidepressive and anxiolytic properties. In general, halogen substitution at position 7 of the benzodiazepine ring (X) caused a decrease in the peak effect and an increase in the duration of action of the anti-TBZ activity as substituents increased in molecular size. Compound **10a** (X = H, ED_{50} = 0.26 mg/kg) was the most active one when 1-h pretreatment was used, followed by **10c** (X = F, 0.39 mg/kg), **10f** (X = Cl, 0.48 mg/kg), and **10i** (X = Br, 0.76 mg/kg). However, **10f'** and **10i** preserved obvious activities even at 24 h after administration, their respective ED_{50} values being 1.47 and 1.26 mg/kg in contrast to no effects with **10a** or **10c** at a dose of 32 mg/kg. The NCH_3 compounds behaved in the same manner. The *N*-(ethoxycarbonyl)piperidine **28** and *N*-acetylpiperidine **30** series were also active but weaker in potency.

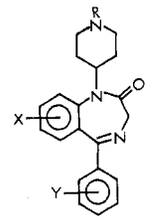
Benzodiazepine derivatives without halogen substituents have been shown to lack antianxiety properties.[1] This rule applies to our **7a** and **10a**. Introduction of F at the X position failed to increase the activity; **10c** as well as **10a** showed neither anti-PTZ nor anticonflict effects with K_i values in the micromolar range in the [^{3}H]diazepam binding test. However, introduction of Cl (**10f'**) or Br (**10i**) resulted in activities 2–3 times as potent as **10a** in the affinity to benzodiazepine receptors, accompanied by weak but significant in vivo potencies. **10f'** became positive in the anticonflict test with an ED_{50} value of 11.81 mg/kg, po. This compound also showed a very weak but dose-dependent antagonism in the PTZ test, if given orally 4 h prior to PTZ (ED_{50} = 47.17 mg/kg, po). One exception was **7c** (X = F, N = CH_3), which had an ED_{50} value of 25.79 mg/kg in the PTZ test in spite of the large K_i value of 2.42 μM. However, this compound showed no significant activity in the anticonflict test.

According to the structure–activity relationship, the larger the halogen substituent at position 7 was, the longer was the duration of the antidepressive properties and the more active were the antianxiety effects.

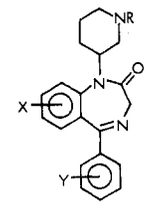
The effects of substituents other than halogen at position 7 (CH_3, **7k**; OCH_3, **7l**; $N(CH_3)_2$, **7m**; NO_2, **7n**) and of halogen substituents at positions other than 7 (8-F, **7o**; 8-Cl, **7p**; 8-CF_3, **7g**; 7,8-Cl, **7r**; 9-Cl, **7s**) were assessed for compounds with F on the ortho position of the phenyl ring (Y = F). The anti-TBZ activity was preserved in compounds receiving any substitution at the 7-position, except for **7n**. **7p** (8-Cl, Y = F) was completely devoid of anti-TBZ activity, but its comparable compound, **7g** (7-Cl, Y = F), was very active. Thus, a substituent at position 8 seemed to be undesirable for the anti-TBZ activity, which reappeared when the substituent was moved to position 9 (**7s**; 9-Cl, Y = F). All these modifications led to marked decreases in anti-PTZ effects.

Antidepressive and anxiolytic properties were affected in opposite ways by the introduction of a halogen substituent at Y. Although the antidepressant activities were little affected by F substitution, the affinities to benzodiazepine receptors were remarkably enhanced as indicated by the great changes in K_i values (**7a,b**, **7c,d**, **7f',g'**, **7i,j**, **10a,b**, **10c,d**, **10f',g'**, **10i,j** etc.). As mentioned above, **7a** and **10a** (X and Y = H) had very low affinities for benzodiazepine receptors (K_i in μM) and no apparent anti-PTZ and anticonflict effects. But **7b** and **10b** (X = H, Y = F) showed significant affinities for the receptor with the respective K_i values of 0.2 and 0.15 μM; they were about 10 times as potent as those of **7a** and **10a**, even though the potentials were still insufficient for producing significant anti-PTZ activity. The manifestation of in vivo efficacies by F substitution on Y were achieved first in **7d** and **10d** (X and Y = F). Compounds **7f**, **7i**, **10f**, and **10i**, having Cl or Br at X and no halogen at Y, could antagonize PTZ-induced seizure with ED_{50} values between 20 and 40 mg/kg. They were more active in inhibiting conflict behaviors (ED_{50} = 8–20 mg/kg, po). Additional F substitution at the Y position yielded potencies in the PTZ test as high as that of chlordiazepoxide, as seen in **7g'**, **7j**, and

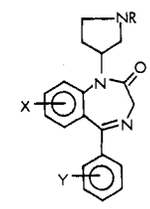
Table I. 1-Azacycloalkyl-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-ones



7, 10, 28, 30



8, 11



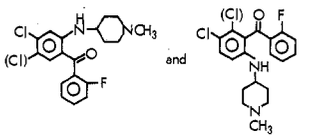
9, 12

compd[a]	X	Y	R	method	yield,[b] %	mp, °C	recrystn[c] solvent	formula	anal.[d]
7a	H	H	CH_3	B	59	157–159	D–E	$C_{21}H_{23}N_3O$	C, H, N
				C[e]	85				
				C[f]	79				
7b	H	2′F	CH_3	B	48	123–125	D–E	$C_{21}H_{22}FN_3O$	C, H, F, N
7c	7-F	H	CH_3	C[e]	84	256–258 dec	F–G	$C_{21}H_{22}FN_3O\cdot HCl$	C, H, Cl, F, N
7c′	7-F	H	CH_3	C[g]	71	223–228 dec	D–I	$C_{21}H_{22}FN_3O\cdot CF_3COOH$	C, H, F, N
7d	7-F	2′-F	CH_3	B	61	146–148	D–E	$C_{21}H_{21}F_2N_3O$	C, H, N, F
				C[g]	54				
				C[f]	85				
7e	7-F	2′-Cl	CH_3	C[e]	53	139–140	D–E	$C_{21}H_{21}ClFN_3O$	C, H, Cl, F, N
7f	7-Cl	H	CH_3	C[e]	71	148–150	D–E	$C_{21}H_{22}ClN_3O$	C, H, Cl, N
7f′	7-Cl	H	CH_3	A	44	250–251 dec	F–G	$C_{21}H_{22}ClN_3O\cdot HCl$	C, H, Cl, N
7g	7-Cl	2′-F	CH_3	B	74	147–149	D–E	$C_{21}H_{21}ClFN_3O$	C, H, Cl, F, N
7g′	7-Cl	2′-F	CH_3	C[e]	74	198–200 dec	H–5% H_2O	$C_{21}H_{21}ClFN_3O\cdot HCl\cdot H_2O$	C, H, Cl, F, N
7h	7-Cl	2′-Cl	CH_3	B	42	146–147	C–E	$C_{21}H_{21}Cl_2N_3O$	C, H, Cl, N
7i	7-Br	H	CH_3	C[e]	51	247–253 dec	D–G	$C_{21}H_{22}BrN_3O\cdot HBr\cdot H_2O$	C, H, N
7j	7-Br	2′-F	CH_3	B	71	170–171	C–E	$C_{21}H_{21}BrFN_3O$	C, H, Br, F, N
7k	7-CH_3	2′-F	CH_3	B	59	139–141	C–E	$C_{22}H_{24}FN_3O$	C, H, F, N
7l	7-OCH_3	2′-F	CH_3	B	68	76–77	E–G	$C_{22}H_{24}FN_3O\cdot i\text{-PrOH}$	C, H, F, N
7m	7-$N(CH_3)_2$	2′-F	CH_3	B	58	142–144	D–E	$C_{23}H_{27}FN_4O$	C, H, F, N
7n	7-NO_2	2′-F	CH_3	*h*	23	199–201	D–E	$C_{21}H_{21}FN_4O_3$	C, H, F, N
7o	8-F	2′-F	CH_3	B	60	200–201	D–E	$C_{21}H_{21}F_2N_3O$	C, H, F, N
7p	8-Cl	2′-F	CH_3	B	22[i]	203–205 dec	D–E	$C_{21}H_{21}ClFN_3O$	C, H, Cl, F, N
7q	8-CF_3	2′-F	CH_3	B	41	121–122	B–E	$C_{22}H_{21}F_3N_3O$	C, H, F, N
7r	7,8-Cl_2	2′-F	CH_3	B	28[i]	168–169	E–G	$C_{21}H_{20}Cl_2FN_3O$	C, H, Cl, F, N
7s	9-Cl	2′-F	CH_3	B	14[j]	181–182	D–E	$C_{21}H_{21}ClFN_3O$	C, H, Cl, F, N
7t	H	4′-F	CH_3	B	80	powder		$C_{21}H_{22}FN_3O$	C, H, F, N
7u	7-Cl	3′-Cl	CH_3	B	71	172–173	C–E	$C_{21}H_{21}Cl_2N_3O$	C, H, Cl, N
7v	7-Cl	H	CH_2CH_2Ph	A	67	217–219 dec	F	$C_{28}H_{28}ClN_3O\cdot C_2H_2O_4$	C, H, Cl, H
10a	H	H	H	D–B	60	281–283 dec	F	$C_{20}H_{21}N_3O\cdot HBr$	C, H, Br, N
				C[e]–E	64				
				A[k]–E	64				
				C[e,l]	69				
10b	H	2′-F	H	D-B	71	166–167	F–I	$C_{20}H_{20}FN_3O$	C, H, F, N
10c	7-F	H	H	C[g]–E	64	121–122	A–E	$C_{20}H_{20}FN_3O$	C, H, F, N
10d	7-F	2′-F	H	C[g]–E	49	150–151	D–E	$C_{20}H_{19}F_2N_3O$	C, H, F, N
				C[f,m]	65				
10d′	7-F	2′-F	H	C[f]–E[n]	61	275–276 dec	E–G	$C_{20}H_{19}F_2N_3O\cdot HCl$	C, H, F, N
10e	7-F	2′-Cl	H	C[e]–E	39	276–278 dec	F–G	$C_{20}H_{19}ClFN_3O\cdot HCl$	C, H, Cl, F, N
10f	7-Cl	H	H	D–B	75	191–192	E–G	$C_{20}H_{20}ClN_3O$	C, H, Cl, N
10f′	7-Cl	H	H			262–265 dec	D–F	$C_{20}H_{20}ClN_3O\cdot HBr\cdot 0.5H_2O$	C, H, Br, Cl, N
10g	7-Cl	2′-F	H	D-A	34	197–199 dec	F–I	$C_{20}H_{19}ClFN_3O$	C, H, Cl, F, N
10g′	7-Cl	2′-F	H	C[e]–E	56	261–262 dec	F–H_2O	$C_{20}H_{19}ClFN_3O\cdot HBr$	C, H, F, N
				C[e,o]	56				
10h	7-Cl	2′-Cl	H	D–A	45	powder		$C_{20}H_{19}Cl_2N_3O$	C, H, Cl, N
				B[k]–E	54				
10i	7-Br	H	H	B–E	48	194–198 dec	E–G	$C_{20}H_{20}BrN_3O$	C, H, Br, N
10j	7-Br	2′-F	H	B–E	51	202–204 dec	E–G	$C_{20}H_{19}BrFN_3O$	C, H, Br, F, N
8	7-Cl	2′-Cl	CH_3	A	44	powder		$C_{21}H_{21}Cl_2N_3O$	C, H, Cl, N
9a	7-Cl	2′-F	CH_3	*p*	42	113–114	C–E	$C_{20}H_{19}ClFN_3O$	C, H, Cl, F, N
9b	7-Cl	2′-Cl	CH_3	B	20	141–143	E	$C_{20}H_{19}Cl_2N_3O$	C, H, Cl, N
9c	7-Cl	2′-F	CH_2Ph	C[e]	77	156–157	D–E	$C_{26}H_{23}ClFN_3O$	C, H, Cl, F, N
11	7-Cl	2′-Cl	H	D–A	34	133–136	E	$C_{20}H_{19}Cl_2N_3O$	C, H, Cl, N
12a	7-Cl	2′-F	H	C[e]–E[q]	62	220–221 dec	A–H_2O	$C_{19}H_{17}ClFN_3O\cdot HBr\cdot 0.5H_2O$	C, H, Br, F, N
12b	7-Cl	2′-Cl	H	D′–A	11	138–140	F	$C_{19}H_{17}Cl_2N_3O\cdot C_2H_2O_4\cdot H_2O$	C, H, Cl, N
28a	H	H	COOEt	A	85	powder		$C_{23}H_{25}N_3O_3$	C, H, N
28b	7-Cl	2′-F	COOEt	B	73	160–161	A–D	$C_{23}H_{23}ClFN_3O_3$	C, H, Cl, F, N
28c	7-Cl	2′-Cl	COOEt	A	58	powder		$C_{23}H_{23}Cl_2N_3O_3$	C, H, Cl, N
30	H	H	$COCH_3$	A	56	208–209	D–G	$C_{22}H_{23}N_3O_2$	C, H, N

[a] All compounds exhibited IR ($\nu_{max}^{CHCl_3}$ ca. 1680 cm^{-1}) and ^1H NMR [($CDCl_3$) δ 3.7–3.8 and 4.7–4.8 (2 H, AB, J = 10 Hz, $COCH_2N$)] spectra consistent with the structures. [b] Isolated yield based on corresponding **2**, **3**, **4**, **5**, and **6**. [c] A = acetone, B = benzene, C = hexane, D = dichloromethane, E = ether, F = methanol, G = 2-propanol, H = tetrahydrofuran, I = ethyl acetate. [d] All compounds were analyzed (C, H, halogens, and N), and the values obtained were within ±0.5% of the theoretical values besides the following data. Calcd (found): **7t** C, 71.77; (71.14%); **8** Cl, 17.63 (16.82); **10h** C, 61.86 (59.66); **10i** C, 60.31 (59.71), Br 20.06 (20.67); **28a** C, 69.88 (70.57); **28b** C, 61.60 (62.23).

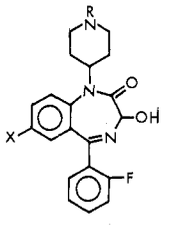
Footnotes to Table I Continued

[e] CF_3COOH was used. [f] PPA was used. [g] H_2SO_4 was used. [h] See Experimental Section. [i] Starting materials were a 3:1 mixture of [j] Crude



2 (X = 6-Cl, R_3 = CH_3, Y = 2-F, ca. 70% purity) was used. [k] **28** was prepared from **20**. [l] **5** (X = Y = R_3 = H) was used as starting material. [m] **5** (X = Y = F, R_3 = H) was used as starting material. [n] $ClCOOCHClCH_3$ was used; otherwise stated ClOOEt was used. [o] **5** (X = 4-Cl, Y = 2-F, R_3 = H) was used as starting material. [p] **12a** (2.9 mmol) was methylated with 37% CH_2O (3.0 mmol) and Raney Ni (5 mL of EtOH) in a solution of MeOH (15 mL) with a H_2 stream at room temperature for 2 h. [q] **9c** was used as starting material. [r] 4-Chloro-*N*-3-pyrrolidinylaniline dihydrochloride was treated as described in ref 2 and the resulting **4** (X = 4-Cl, Y = 2-Cl, R_3 = H) was subjected to ethoxycarbonylation, giving oily **4** (X = 4-Cl, Y = 2-Cl, R_3 = $COOCH_2Ph$, 38%): IR ν_{max}^{film} 3290, 1690, 1620 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.12 (2 H, s, CH_2Ph), 9.1 (1 H, d, J = 6 Hz, NH). This was converted into **12b** by using method A.

Table II. 1,3-Dihydro-5-(2-fluorophenyl)-3-hydroxy-1-(4-piperidinyl)-2*H*-1,4-benzodiazepin-2-ones



34, **35**

compd[a]	X	R	method	yield,[b] %	mp, °C	recrystn[c] solvent	formula	anal.[d]
34a	H	H	F	67	190–192	D–I	$C_{20}H_{20}FN_3O_2$	C, H, F, N
34b	Cl	H	F	57	215–216 dec	F	$C_{20}H_{19}ClFN_3O_2$	C, H, Cl, F, N
35	Cl	CH_3	*e*	43	197–198	D–I	$C_{21}H_{22}ClFN_3O_2$	C, H, Cl, F, N

[a] All compounds exhibited IR ($\nu_{max}^{CHCl_3}$ 3450–3460, 1670–1680 cm^{-1}) and ^1H NMR [($CDCl_3$) δ 4.9–5.0 (1 H, s, NC(OH)*H*CO)] spectra consistent with the structures. [b] Yield based on the corresponding **26**. [c] See footnote *c* in Table I. [d] All compounds were analyzed (C, H, halogens, and N), and the values obtained were within ±0.3% of the theoretical values. [e] See Experimental Section.

10j. In the conflict behavior test, **7g′** and **7j** were much more active than CDP. Further increases in anti-PTZ potencies were achieved by introducing Cl at Y, as indicated by comparisons of the anti-PTZ activities between **7e**, **7h**, **10e**, and **10h** and their corresponding F-substituted **7d**, **7g′**, **10d**, and **10g′**. In contrast, obvious decreases in antidepressive effects were noted by this modification; i.e., anti-TBZ effects and inhibitory potentials for NE uptake of the Cl-substituted compounds were about $^1/_{10}$ to $^1/_{30}$ as active as those of the corresponding F-substituted compounds. From these data, it can be concluded that the anticonvulsant and antianxiety effects were readily augmented by the substitution at Y in the order of H << F < Cl, while the order for the anti-TBZ effect and the preventive effect on NE uptake was H = F >> Cl. These orders were also found for a limited number of compounds having a pyrrolidine ring at position 1 (**9a,b** and **12a,b**).

1-(3-Piperidinyl)-1,4-benzodiazepin-2-one derivatives with Cl substituents at the X and Y positions (**8**, **11**) were tested. In comparison with their corresponding 4-piperidinyl derivatives (**7h**, **10h**), antidepressive potencies were found to be significantly reduced, while antianxiety effects were well preserved. This finding suggests that the three carbon atoms between N at position 1 and N of the piperidine ring may be suitable for producing the antidepressant activity.

The introduction of OH at position 3 led to an apparent reduction in the antidepressive properties, while the anxiolytic effects were not influenced (**35**, **34b**).

The test compounds had weak acute toxicities. The most striking structural feature influencing toxicity was CH_3 substitution for R; the toxicity increased considerably when the NH was replaced by NCH_3. However, even the most toxic compound so far tested (**7d**) had an LD_{50} value very close to those of conventional tricyclic antidepressants.

With benzodiazepine compounds, antianxiety with antidepressant activities have been reported for triazolobenzodiazepine derivatives, alprazolam,[11] adinazolam,[12] and U-43,465F.[13] Since the antianxiety effect of these triazolobenzodiazepines generally predominated over the antidepressive properties, our compounds, i.e., **7a**, **10a**, **7d**, **10d**, etc., should be more suitable for clinical use as potent antidepressants.

In conclusion, 1-(4-piperidinyl)- or 1-(3-pyrrolidinyl)-1,4-benzodiazepin-2-ones appear to have potent antidepressant properties together with antianxiety properties. Halogen substitution, the most conventional modification for increasing the antianxiety effect of benzodiazepines, yielded interesting changes in both antidepressive and antianxiety properties. Following introduction of a halogen at position 7 of the 1,4-benzodiazepine ring (X), the antidepressive properties, assessed by antagonism to TBZ-induced ptosis in mice, showed decreases in their peak effects but became long lasting. On the other hand, the antianxiety properties were obviously enhanced. Further increases in the anti-PTZ effect were attained with a halogen substituent in the ortho position of the phenyl ring at position 5 (Y). The antidepressant properties were greatly reduced when Cl was introduced to Y. The experiment on uptake inhibition of [^{3}H]norepinephrine into rat brain synaptosomes revealed that the secondary amine

(11) Feighner, J. P.; Aden, G. C.; Fabre, L. F.; Rickels, K.; Smith, W. T. *J. Am. Med. Assoc.* **1983**, *249*, 3057.
(12) Hester, J. B., Jr.; Rudzik, A. D.; Von Voigtlander, P. F. *J. Med. Chem.* **1980**, *23*, 392.
(13) Von Voigtlander, P. F.; Puech, A. F. *Arch. Int. Pharmacodyn. Ther.* **1983**, *266*, 60.

Table III. Biological Activities (Antidepressive Effects)

no.	TBZ[a] ED_{50}, mg/kg (95% CL) po[c]	TBZ[a] ED_{50}, mg/kg (95% CL) sc[d]	^3H[NE] IC_{50}, μM
7a	0.38 (0.14–0.84)	0.26 (0.15–0.47)	
7b		0.21 (0.11–0.33)	
7c			
7c′	1.41 (0.85–2.44)		
7d	1.62 (0.78–4.25)	0.27 (0.15–0.45)	53.7
7e	59.34 (36.74–148.6)		
7f	0.74 (0.41–1.22)	0.85 (0.52–1.14)	
7f′	0.58 (0.33–0.96)		
7g	2.13 (1.25–3.55)	1.30 (0.68–2.56)	
7g′	1.12 (0.45–1.94)		
7h	26.14 (14.24–49.03)	23% at 50 mg/kg[e]	39.9
7i	2.88 (1.36–5.51)		
7j	3.85 (2.49–5.93)	2.45 (1.46–4.64)	
7k		0.84 (0.41–1.52)	
7l		1.24 (0.68–2.39)	
7m		0.70 (0.31–3.20)	
7n		17.74 (10.85–30.23)	
7o		0.94 (0.57–1.43)	
7p		34% at 50 mg/kg	
7q		NE at 50 mg/kg[f]	
7r		64% at 50 mg/kg	
7s		2.66 (1.57–4.41)	
7t		53% at 50 mg/kg	
7u		19.26 (8.32–172.7)	
7v		7.88 (4.38–14.96)	
10a	0.26 (0.14–0.45)	0.06 (0.02–0.11)	0.029
10b		0.07 (0.05–0.11)	
10c	0.39 (0.19–0.65)		0.059
10d	0.38 (0.16–0.70)		0.079
10d′			
10e	13.48 (6.73–27.70)		0.877
10f	0.48 (0.24–0.96)	0.12 (0.06–0.22)	
10f′	0.48 (0.27–0.79)		0.172
10g	0.67 (0.46–1.01)	0.08 (0.04–0.12)	
10g′	0.76 (0.51–1.12)		0.118
10h	9.59 (5.52–15.08)	9.94 (2.40–33.19)	0.562
10i	0.76 (0.40–1.47)		0.402
10j	5.37 (3.43–8.29)		0.288
8	21% at 100 mg/kg		
9a	3.69 (2.27–6.51)		
9b	61% at 100 mg/kg		
9c	58% at 50 mg/kg		
11	57% at 50 mg/kg		
12a	0.40 (0.23–0.66)	28.92 (9.43–305.1)	
12b	34% at 50 mg/kg	12.57 (6.58–21.78)	
28a		0.65 (0.38–1.11)	
28b		67% at 50 mg/kg	
28c		16% at 50 mg/kg	
30		3.25 (1.76–6.06)	
34a		0.23 (0.10–0.49)	
34b	6.51[g]		
35	9.79 (4.58–19.22)		
IMP[h]	2.14 (1.32–3.37)	0.80 (0.40–1.96)	0.085
CDP[i]			

[a] Antagonism of tetrabenazine-induced ptosis in mice. ED_{50} values and their 95% confidence limits were calculated by regression analysis (n = 8). [b] Uptake inhibition of [^3H]norepinephrine into rat brain synaptosomes. Presumed IC_{50} values were obtained by graphical interpolation. [c] Oral administration. [d] Subcutaneous administration. [e] Percent antagonism at that dose. [f] No effect at that dose. [g] Estimated ED_{50}. 95% confidence limits not given because of poor dose dependency. [h] Imipramine. [i] Chlordiazepoxide.

function of the azacyclic ring at position 1 was essential for the production of the antidepressant properties.

Although animal data on antianxiety effects correlate well with clinical dosages, there is no conclusive antidepressant test for predicting effective clinical dosages. Irrespective of differences in preclinical potencies, daily clinical dosages of each known antidepressant are mostly in the same range (30–100 mg/man per day). This also applies to our drug candidate. Furthermore, obviously beneficial effects in depressive patients are usually produced following long-term treatments with an antidepressant drug. Thus, if the antianxiety effect of our antidepressant candidate is as potent as CDP, accompanying effects such as sedation or muscle relaxation will become so potent as to overwhelm the essential antidepressive effect following long-term treatments with large doses. Therefore, we think the antianxiety effect of our antidepressant candidate should not exceed that of CDP, the weakest minor tranquilizer. This rationale led us to select compound **10d** as the most suitable candidate from the new series for development into an antidepressant drug.

Experimental Section

Melting points were determined on a Yanagimoto micromelting apparatus and are uncorrected. IR spectra were recorded in $CHCl_3$ solution by a Hitachi 260-10 IRS spectrophotometer. Wavenumbers are expressed in reciprocal centimeters. NMR spectra were taken in $CDCl_3$ solution on a Varian EM-390 or T-60 spectrophotometer. Chemical shifts are expressed as δ values (parts per million) from tetramethylsilane. Column chromatography was conducted with use of silica gel (E. Merck, 70–230-mesh ASTM) and aluminum oxide (E. Merck, Standarisiert). Silica gel GF and aluminum oxide F254 (E. Merck) were used for analytical thin-layer chromatography (TLC). In cases where products were isolated by solvent extraction, the procedure generally followed was to extract the aqueous layer with two to three portions of the indicated solvent, then wash the organic layer with saturated NaCl–H_2O or H_2O, and dry it over Na_2SO_4 or $MgSO_4$.

Method A. To a stirred solution of a 2-acyl-*N*-azacycloalkylaniline hydrochloride **2** or **3** (R_3 = CH_3, CH_2CH_2Ph, 6 mmol) in CH_3CN (20 mL), prepared by adding of 1 equiv of HCl gas–MeOH to a solution of **2** or **3** in CH_3OH and concentrating the mixture, was added chloroacetyl chloride (6 × 2 mmol) and the solution was refluxed for 1–20 h. TLC (Al_2O_3GF, $CHCl_3$–AcOEt = 5:1) was used to check for completion of the reaction. After removal of the excess reagent and solvent under reduced pressure, the resulting chloroacetyl derivative hydrochloride **13** or **14** was dissolved again in CH_3CN (50 mL) containing NaI (6 × 3 mmol) and the solution was warmed at 70 °C for 2 h. The exchange of the chloroacetyl for the iodoacetyl was checked by monitoring with TLC (Al_2O_3GF, CH_2Cl_2) and ^1H NMR [($CDCl_3$) ca. δ 4.1 and 3.9 (AB q, J = 12 Hz, $COCH_2Cl$), ca. 3.4 and 3.7 (AB q, J = 10 Hz, $COCH_2I$)]. After the solution cooled, $(NH_4)_2CO_3$ (6 g) was added and the mixture was stirred at room temperature for 10 days. In an alternate procedure, prior to adding $(NH_4)_2CO_3$, CH_3CN was evaporated, H_2O was added, and the mixture was extracted with CH_2Cl_2. Evaporation of the solvent and addition of acetone gave the iodoacetyl derivative hydriodides of **13** or **14**. These were dissolved in CH_3CN (50 mL) containing $(NH_4)_2CO_3$ (6 g), and the mixture was warmed in a sealed vessel at 70 °C for 10 h. After the mixture cooled, the solvent was evaporated, H_2O was added, and the mixture was extracted with CH_2Cl_2. The residue was purified on Al_2O_3 (60 g, benzene and benzene containing 1–5% EtOAc), giving **7** or **8**.

Method B. A solution of a 2-acyl-*N*-azacycloalkylaniline **2** or **4** (R_3 = CH_3) (10 mmol) and phthaloylglycyl chloride (10 × 2 mmol) in CH_3CN (30 mL) was refluxed for 20–140 h. The reaction was monitored by TLC (Al_2O_3 GF, CH_2Cl_2–AcOEt = 5:1). The solvent was evaporated and then ice–2 N NaOH was added and the mixture was stirred at room temperature for 30 min and extracted with CH_2Cl_2. The extract was purified on Al_2O_3 (100 g, CH_2Cl_2) to obtain analytically pure **16** or could be directly used for the next step. The phthaloylacetyl derivative **16** or **17** was dissolved in 95% EtOH (60 mL) containing 80% $NH_2NH_2·H_2O$ (10 × 3 mmol) and the solution was refluxed for 0.5–2 h. The solution was concentrated and the precipitate was filtered off. After concentration of the filtrate, the residue was purified on Al_2O_3 (50 g, benzene, benzene containing 2–5% AcOEt and benzene containing 5% MeOH), giving **7** or **9** and the corresponding **15** as the more polar fraction.

Method C. A solution of a 2-acyl-*N*-azacycloalkylanilineimine **5** or **6** (R_3 = CH_3, CH_2Ph) (0.2 mol) in EtOH (700 mL) containing glycine ethyl ester hydrochloride (0.2 × 1.2 mol) and AcOH (0.2 mol) was refluxed for 3 h. After removal of the solvent, the residue was made alkaline with dilute NH_4OH and extracted with CH_2Cl_2.

Table IV. Biological Activities (Minor Tranquilizing Effects)

no.	PTZ[a] ED_{50}, mg/kg (95% CL) po	PTZ[a] ED_{50}, mg/kg (95% CL) sc	conflict[b] ED_{50}, mg/kg, po (95% CL)	[^{3}H]DZ[c] K_2, μM
7a	NE at 50 mg/kg	NE at 10 mg/kg		2.3
7b	37.5% at 50 mg/kg			0.2
7c	25.79 (17.62–35.90)		20% at 16 mg/kg	2.42
7c′	29.96 (18.28–94.90)			
7d	26.92 (18.90–31.75)	5.97 (4.41–8.50)	11.36 (6.66–25.48)	0.094
7e	5.20 (3.09–8.15)			0.014
7f		50% at 20 mg/kg		
7f′	29.90 (21.77–41.13)		8.40 (3.78–14.34)	0.49
7g	2.70 (1.66–3.96)			
7g′	5.28 (3.95–6.96)	0.71 (0.37–1.11)	3.67 (1.71–7.11)	0.042
7h	3.24 (2.02–4.87)	1.69 (0.78–4.85)		0.021
7i	21.36 (13.74–37.28)		17.35[d]	0.415
7j	1.61 (1.07–2.31)	0.79 (0.50–1.24)	1.23 (0.79–2.58)	0.049
7k		NE at 10 mg/kg		0.26
7l		NE at 10 mg/kg		1.26
7m		NE at 10 mg/kg		8.3
7n		NE at 10 mg/kg		8.3
7n		NE at 10 mg/kg		24
7o	NE at 50 mg/kg	NE at 10 mg/kg		0.44
7p	NE at 50 mg/kg	NE at 10 mg/kg		0.69
7q		NE at 20 mg/kg		17
7r	NE at 50 mg/kg	NE at 10 mg/kg		0.80
7s		NE at 10 mg/kg		4.7
7t		NE at 10 mg/kg		68
7u		NE at 10 mg/kg		23
7v		NE at 10 mg/kg		1.6
10a	NE at 200 mg/kg	NE at 10 mg/kg	20% at 32 mg/kg	1.3
10b	NE at 50 mg/kg	NE at 10 mg/kg		0.15
10c	12.5% at 50 mg/kg		60% at 32 mg/kg	2.0
10d	32.11 (2.137–46.27)		50% at 16 mg/kg	0.15
10d′	27.55 (19.37–42.22)			
10e	29.44 (20.35–43.04)			0.012
10f	36.45 (26.12–49.63)	NE at 10 mg/kg		
10f′	NE at 50 mg/kg		11.81 (6.15–33.94)	0.35
10g	11.39 (8.58–15.78)	8.52 (5.28–16.64)		
10g′	14.17 (9.91–20.25)		6.51 (4.22–10.04)	0.036
10h	12.89 (8.84–17.94)			0.017
10i	38.78 (27.95–58.20)			0.57
10j	2.70 (2.00–3.91)		4.54[d]	0.033
8	12.03 (7.92–18.99)			0.016
9a	1.10 (0.72–1.72)			0.2
9b	5.78 (3.95–9.41)			0.05
9c	1.52 (0.86–3.18)			0.27
11	16.17 (11.71–22.28)			0.013
12a	20.12 (14.27–32.17)		60% at 8 mg/kg	0.73
12b	9.63 (6.42–15.50)			0.027
28a		NE at 10 mg/kg		2.3
28b	9.09 (5.81–13.95)	11.14 (5.96–26.04)		0.038
28c	1.95 (1.07–3.96)			0.02
30		NE at 10 mg/kg		1.5
34a		NE at 10 mg/kg		0.36
34b	12.50 (8.35–18.59)		8.80 (5.15–24.51)	0.14
35	2.41 (1.70–3.40)			0.074
IMP				
CDP	2.70 (1.66–3.96)		6.49 (3.10–10.11)	0.202

[a] Antagonism of pentylenetetrazol-induced convulsion in mice. ED_{50} values and their 95% confidence limits were calculated by the probit method (n = 8). [b] Anticonflict effects in rats. ED_{50} and 95% confidence limits were calculated by the probit method (n = 5–8). [c] Displacing potential to [^{3}H]diazepam binding in rat cerebral cortex. Presumed K_i values were calculated with use of IC_{50} values obtained by graphical interpolation. [d] Estimated ED_{50}. 95% confidence limits not obtained because of poor dose dependency.

The extract was crystallized from Et_2O–hexane to obtain analytically pure iminoglycine ester **18** or **19** or used directly for the next step.

Cyclization Using CF_3COOH. An iminoglycine ester **18** or **19** (0.2 mol) was dissolved in CF_3COOH (600 mL) and the solution was refluxed for 20 h. The solvent was evaporated under reduced pressure, benzene was added to the residue, and the solvent was again evaporated to remove residual CF_3COOH. Addition of benzene to the resulting syrup followed by Et_2O generally gave crystalline **7** or **9**·CF_3COOH. The crystalline or oily **7** or **9**·CF_3COOH was alkalized with ice–concentrated NH_4OH (20 mL) and extracted with CH_2Cl_2. Crystallization of the extract gave pure **7** or **9** or the corresponding salt. The filtrate of **7** or **9**·CF_3COOH was concentrated and the residue was made alkaline with ice–concentrated NH_4OH and extracted with CH_2Cl_2. From the extract, **2** or **4** was recovered in 5–10% yield. When the starting material was **5** (R_3 = H), CF_3COOH was evaporated and the residue was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed successively with 5% AcOH and H_2O. The combined acidic layer was made alkaline with concentrated NH_4OH and extracted with CH_2Cl_2. Purification of the extract gave **10**. The above-mentioned CH_2Cl_2 layer was washed with dilute NH_4OH and concentrated to recover **2** (R_3 = H) in 10–20% yield.

Cyclization Using H_2SO_4. An iminoglycine ester **18** or **19** (1.5 mol) was dissolved in concentrated H_2SO_4 (30 mL) and the mixture was warmed 90 °C for 13 h with stirring. After cooling, the mixture was poured onto ice (150 g) and made alkaline with NaOH (49 g) and ice–H_2O (200 mL). The mixture was extracted with benzene. Crystallization of the extract gave **7** or **9**. After concentration of the mother liquor, CF_3COOH (0.3 mL) and Et_2O

Table V. Biological Activities (Acute Toxicities)

no.	mouse LD_{50},[a] mg/kg, po (95% CL)
7a	425 (385–472)
7d	267 (230–328)
7f'	548 (510–588)
7g'	489 (425–553)
10a	1239 (1113–1386)
10d	939 (805–1213)
10f'	1393 (1293–1506)
10g'	1480 (1306–1666)
IMP[b]	310 (293–330)
CDP[c]	877[d]

[a] LD50 values were calculated by the probit method (n = 10). 95% confidence limits were included parentheses. [b] Imipramine. [c] Chlordiazepoxide. [d] Estimated LD_{50} obtained by the up and down method (n = 5).

(30 mL) were added to the residue. The crystallized **7** or **9**·CF_3COOH were filtered off and converted to free **7** or **9** as described above. From the mother liquor of **7** or **9**·CF_3COOH, **2** or **4** were recovered in a manner similar to the above.

Cyclization Using PPA. A mixture of an iminoglycine ester **18** (0.1 mol) and PPA (250 g) was warmed at 100 °C (bath temperature) with stirring for 4 h. After cooling, ice (500 g) and concentrated NH_4OH (320 mL) were added, and the mixture was extracted with CH_2Cl_2. Ether was added to the extract and the resulting crude **7** was separated and recrystallized from CH_2Cl_2–ether to obtain pure **7**. Purification of the mother liquor in a similar manner after its conversion into **7**·CF_3COOH gave additional pure **7**.

Method D. To a refluxing solution of 2-acyl-*N*-azacycloalkylaniline **2** or **3** (R_3 = CH_3, 15 mmol) in dichloroethane (50 mL) was added dropwise ethyl chloroformate (15 × 3 mmol) over a period of 10 min and the solution was refluxed for 1 h. To complete the reaction, $NaHCO_3$ (15 mmol) was added to the solution and the mixture was refluxed a further 1 h. TLC (SiO_2GF, $CHCl_3$–CH_3OH = 5:1) was used to check for the completion of the reaction. After cooling, ice–H_2O was added, the benzene layer was separated, and the H_2O layer was extracted with benzene. The combined benzene extract was crystallized to obtain a pure sample of **20** or **21** or could be directly used for the next step. An *N*-ethoxycarbonyl derivative **20** or **21** was dissolved in concentrated HCl–H_2O (1:1, 50 mL) and the solution was refluxed for 20 h. TLC (Al_2O_3GF, CH_2Cl_2) was used to check for completion of the reaction. The solution was concentrated in vacuo, giving crude **22** or **23**, which was crystallized to obtain a pure sample or used directly for the next step. To a stirred mixture of **22** or **23** (15 mmol) in dioxane (50 mL) containing 2 N NaOH (19 mL) was added dropwise a solution of benzyl chloroformate (15 × 2.2 mmol) in dioxane (10 mL) with ice-cooling. The mixture was stirred at room temperature for 1.5 h. TLC (Al_2O_3GF, CH_2Cl_2, or SiO_2GF, $CHCl_3$–CH_3OH = 5:1) was used to check for completion of the reaction. H_2O was added and the mixture was extracted with benzene. The extract was purified on Lobar column B (CH_2Cl_2–AcOEt = 10:1), giving crude **24** or **25**. These were converted to **26** or **27** by use of method A or B. Compound **26** or **27** (6 mmol) was dissolved in a solution of CH_2Cl_2 (50 mL) and CH_3NO_2 (50 mL) containing $AlCl_3$ (6 × 6 mmol) and anisole (6 × 6 mmol) and the mixture was stirred at room temperature for 20 h. Ice–H_2O was added and the mixture was washed with Et_2O. The Et_2O layer was washed with dilute HCl. The combined acidic layer was made alkaline with 2 N NaOH and the mixture was extracted with Et_2O. Crystallization of the extract gave **10** or **11**. Alternatively, compound **26** or **27** (40 mmL) was dissolved in CF_3COOH (53 mL) and the solution was refluxed for 2 h. After removal of the solvent in vacuo, ice–H_2O was added and the mixture was extracted with Et_2O. The H_2O layer was made alkaline with dilute NH_4OH and extracted with CH_2Cl_2. The extract was crystallized, giving **10** or **11**.

Method E. Procedure Using Ethyl Chloroformate. To a refluxing solution of 1-(1-methyl-4-piperidinyl)- or 1-(1-benzyl-3-pyrrolidinyl)-1,4-benzodiazepin-2-one (**7**, R_1 = CH_3; **9**, R_1 = CH_2Ph) (50 mmol) in dichloroethane (150 mL) was added dropwise ethyl chloroformate (50 × 3 mmol) over a period of 5 min and the solution was refluxed for 0.5 h. After cooling, the solution was washed with dilute HCl and the organic layer was evaporated to obtain crude **28** or **29**. The acidic layer was made alkaline with concentrated NH_4OH and extracted with CH_2Cl_2. From the extract, **7** (R_1 = CH_3) was recovered in a yield of less than 5%. Compound **28** or **29** was dissolved in methanesulfonic acid (41 mL) containing tetrahydrothiophene (48 × 5 mmol) and the mixture was warmed at 120 °C with stirring for 0.5 h. After cooling, ice–H_2O was added and the mixture was washed with CH_2Cl_2. The acidic layer was made alkaline with concentrated NH_4OH and extracted with CH_2Cl_2. Crystallization of the extract gave **10** or **12**.

Procedure Using α-Chloroethyl Chloroformate. To a stirred solution of **7** (R_1 = CH_3, 0.12 mol) in dichloromethane (230 mL) was added dropwise a solution of α-chloroethyl chloroformate (14.7 mL, 0.13 mol) in dichloromethane (70 mL) at −5 °C over 15 min and the solution was refluxed for 1.5 h. After cooling, CH_3OH (100 mL) was added with stirring at 20 °C and the solution was refluxed for 2 h with evolution of CO_2. After removal of the solvent, the residue was dissolved in CH_3OH (150 mL), and the solution was decolorized with active charcoal and concentrated. Purification of the residue by crystallization gave **10**·HCl.

1,3-Dihydro-5-(2-fluorophenyl)-1-(1-methyl-4-piperidinyl)-7-nitro-2*H*-1,4-benzodiazepin-2-one (7n). To a stirred solution of 1,3-dihydro-5-(2-fluorophenyl)-1-(1-methyl-4-piperidinyl)-2*H*-1,4-benzodiazepin-2-one (**7b**; 2.1 g, 5.9 mmol) in concentrated H_2SO_4 (10 mL) was added dropwise a solution of KNO_3 (571 mg, 5.9 mmol) in concentrated H_2SO_4 (5 mL) at −8 °C. The solution was allowed to stand at −5 °C for 6 h and then at room temperature for 15 h. The reaction mixture was poured onto ice (200 g), made alkaline with concentrated NH_4OH, and extracted with CH_2Cl_2. The extract was chromatographed on Al_2O_3 (200 g, CH_2Cl_2 and EtOAc). The eluate of EtOAc was collected and recrystallized, giving **7n** (544 mg).

1,3-Dihydro-1-[1-(ethoxycarbonyl)-4-piperidinyl]-5-phenyl-2*H*-1,4-benzodiazepin-2-one (28) from *N*-[1-(Ethoxycarbonyl)-4-piperidinyl]-2-benzoylaniline (20). Compound **20** was treated according to method A or B. Crude **28** was chromatographed on a Lobar column (EtOAc) or crystallized from the appropriate solvent to obtain a pure sample of **28**.

1-(1-Acetyl-4-piperidinyl)-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (30). 2-Benzoyl-*N*-(4-piperidinyl)aniline (**22**; X = Y = H, 2.8 g) was dissolved in Ac_2O (5 mL) and the solution was kept at room temperature for 0.5 h. Ice and K_2CO_3 were added, and the mixture was stirred for 0.5 h and extracted with ether. The extract was chromatographed on Lobar column B (EtOAc), giving oily **31** (3 g, X = Y = H). Compound **31** was treated by use of method A, the extract was chromatographed on Al_2O_3 (CH_2Cl_2), and the eluate was crystallized to obtain pure **30**.

Method F. To a solution of 1-[1-(benzyloxycarbonyl)-4-piperidinyl]-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (**26**; 8.9 mmol) in AcOH (50 mL) was added 30% H_2O_2 (3.5 mL, 8.9 × 4.5 mmol) and the solution was warmed at 65 °C for 16 h. TLC (SiO_2GF, CH_2Cl_2–EtOAc = 1:1) was used to check for completion of the reaction. After cooling, $NaHSO_3$ (3.5 g) was added and the solution was concentrated to about one-fourth volume. Ice and concentrated NH_4OH were added, and the mixture was extracted with CH_2Cl_2. The extract was purified on SiO_2 (15 g, EtOAc–benzene = 1:1), giving oily *N*-oxide **32**: IR $\nu_{max}^{CHCl_3}$ 1680 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.6 (2 H, s, $COCH_2N\rightarrow O$), 5.1 (2 H, s, OCH_2Ph). A solution of compound **32** (8.9 mmol) in Ac_2O (25 mL) was heated at 140 °C for 1 h and then excess Ac_2O was removed under reduced pressure. The residue was purified on SiO_2 (10 g, CH_2Cl_2–EtOAc = 5:1), giving the oily 3-acetoxy derivative **33**: IR $\nu_{max}^{CHCl_3}$ 1610, 1690 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.3 (3 H, s, $COCH_3$), 5.1 (2 H, s, O CH_2Ph), 5.90 (1 H, s, NC(OAc)HCO). Compound **33** was treated with $AlCl_3$ and anisole as in method D, giving **34**.

7-Chloro-1,3-dihydro-5-(2-fluorophenyl)-3-hydroxy-1-(1-methyl-4-piperidinyl)-2*H*-1,4-benzodiazepin-2-one (35). To a stirred solution of 7-chloro-1,3-dihydro-5-(2-fluorophenyl)-3-hydroxy-1-(4-piperidinyl)-2*H*-1,4-benzodiazepin-2-one (**34b**; 900 mg, 2.32 mmol) in CH_3CN (270 mL) were added K_2CO_3 (321 mg, 2.32 mmol) and a solution of $(CH_3)_2SO_4$ (0.22 mL, 2.32 mmol) in CH_3CN (30 mL), and the mixture was stirred at room temperature for 0.5 h. The precipitate was filtered off and the filtrate was

concentrated in vacuo. Ice and concentrated NH_4OH were added, and the mixture was extracted with CH_2Cl_2. The extract was purified on SiO_2 (10 g, CH_2Cl_2 and CH_2Cl_2 containing 10% MeOH). Crystallization of the eluate gave **35** (400 mg).

Antagonism of Tetrabenazine-Induced Ptosis in Mice. Male DS strain mice, weighing 20–27 g, were randomly assigned to groups of five each. Test compounds were dissolved in physiological saline or suspended in arabic gum solution and orally or subcutaneously administered at 0.1 mL/10 g of body weight. A dose of 50 mg/kg of tetrabenazine (TBZ) was subcutaneously injected 1, 4, or 24 h after the test compounds. The animals were scored for ptosis 1 h after TBZ administration according to the following scale: normal = 1, slightly closed = 2, half closed = 3, 100% closed = 4. Observed scores were subjected to ridit transformation[14] and doses causing 50% prevention of the response were calculated by regression analysis.

Antagonism of Pentylenetetrazol-Induced Convulsion in Mice. Eight male ddy strain mice, weighing 20–24 g, were challenged with a dose of 125 mg/kg sc of pentylenetetrazol (PTZ) 1 h after oral or subcutaneous administration of the test compounds. The dose required to prevent tonic convulsion and death in 50% of the animals during a 2-h observation period was calculated by the probit method.[15]

Anticonflict Effect in Rats. To more directly evaluate rat antianxiety activities, their anticonflict behavior was observed with selected compounds by using the method described by Geller and Seifter.[16] Groups of five to eight Wistar rats were trained to press a lever for a food reward on a variable interval of a 60-s schedule. When the lever pressing was established, a tone stimulus of 3-min duration was presented every 15 min as a signal informing every fifth lever pressing would be rewarded with a food pellet. After this behavioral contingency was established, conflict was introduced by punishment with a 60-Hz electroshock through the grid floor at every fifth lever response during the tone period. A dose was determined as positive when the number of shocks exceeded 12 or the shock ratio was more than 4 during four consecutive tone periods. Doses for 50% suppression of the conflict behavior were calculated by the probit method.

Acute Toxicity in Mice. Male DS strain mice, weighing 20–26 g, were employed to assess the oral LD_{50} values for selected compounds. The mortality was determined with groups of 10 mice on the next day after the treatment. LD_{50} values were calculated by the probit method or the up and down method of Brownlee et al.[17]

Uptake Inhibition of [^{3}H]Norepinephrine into Rat Brain Synaptosome. The procedure was mainly based on the method of Randrup and Braestrup.[9] Synaptosomal P2 fractions were prepared from the cerebral cortex of male Wistar rats. The fraction was incubated with [^{3}H]norepinephrine (NE) at 37 °C for 20 min in the presence of a test compound in five to six different concentrations. The reaction was terminated by dilution with Krebs–Henseleit solution and then by filtration with Whatman glass fiber filter (GF/C). The obtained radioactive sample was dissolved in scintillation fluid and the radioactivity was counted with a scintillation counter. IC_{50} values were calculated as the concentration of the test compound inhibiting the uptake of [^{3}H]NE by 50%.

Displacing Potential to [^{3}H]Diazepam Binding. The cerebral cortex homogenates of Wistar strain rats were used for the binding assay. Our procedure was mostly based on the method of Möhler and Okada.[10] Tissue homogenate, a test compound, and 2 nM [^{3}H]diazepam (DZ) were incubated at 0 °C for 60 min. After termination of the incubation, the radioactive sample, trapped on Whatman glass filter (GC/F), was dissolved in scintillation fluid and the radioactivity was measured with a scintillation counter. K_i values were calculated from the following equation: $K_i = IC_{50}/(1 + (L/K_d))$, where IC_{50} is the concentration of the test compound causing 50% inhibition of specific binding of the radioactive ligand and L and K_d are the concentration and the dissociation constant of the ligand, respectively.

Acknowledgment. We thank Dr. H. Otsuka, Managing Director of these laboratories, and Dr. T. Komeno, Director of these laboratories, for their encouragement and permission to publish this work. We also thank I. Sakaguchi, C. Ishibashi, and N. Mugitani for their technical assistance.

Supplementary Material Available: Summarized spectral data characteristic for the structures of intermediates **13**, **14**, and **16–29** and melting point and combustion data for crystalline intermediates and byproduct **15** (9 pages). Ordering information is given on any current masthead page.

(14) Bross, I. D. J. *Biometrics* **1958**, *14*, 18.
(15) Bliss, C. J. Q. *J. Year Book Pharm.* **1938**, *11*, 192.
(16) Geller, I.; Seifter, J. *Psychopharmacology* **1960**, *1*, 482.
(17) Brownlee, K. A.; Hodges, J. L., Jr.; Rosenblatt, M. *J. Am. Stat. Assoc.* **1953**, *48*, 262.