Benzodiazepine Receptor Binding and Anticonflict Activity in a Series of 3,6-Disubstituted Pyridazino[4,3-*c*]isoquinolines Devoid of Anticonvulsant Properties

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A series of 3,6-disubstituted pyridazino[4,3-*c*]isoquinolines were synthesized and tested for their ability to inhibit the binding of [^{3}H]diazepam to rat brain receptors in vitro. Compounds bearing a phenyl, 4-methoxyphenyl, or methyl group at position 3 and a dialkylamino group at position 6 showed the highest affinity in the binding assay and were subsequently evaluated for their anticonflict and anticonvulsant effects. All of these compounds (**5a–l** and **5q**) were active in the Vogel rat conflict procedure, but none prevented convulsions in mice induced either by metrazol or bicuculline. 3-Phenyl-6-pyrrolidinylpyridazino[4,3-*c*]isoquinoline (**5d**) with a K_i = 11.4 nM in the binding assay exhibited the best potency in the anticonflict assay (MED 5 mg/kg ip) and did not produce neuromuscular impairment at the highest dose tested (50 mg/kg ip).

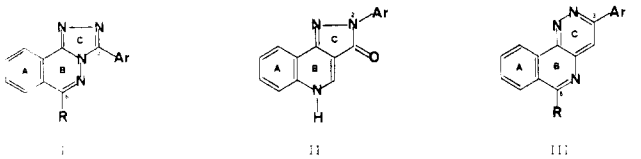
The search for antianxiety agents that are devoid of the side effects associated with the benzodiazepines (BZs) has led to the discovery of various non-BZ classes of compounds with high in vitro activity in the BZ binding assay. Various members of these classes are being actively investigated as potential anxiolytics.[1] It is not apparent, however, how structurally unrelated chemical entities can interact with the same BZ receptor, nor is it understood why slight structural modifications can produce agonists and antagonists from the same parent compound.[2] The discovery of the high BZ binding site affinity of several 3-aryl-1,2,4-triazolo[3,4-*a*]phthalazines (I)[3] and 2-arylpyrazolo[4,3-*c*]quinolin-3(5*H*)-ones (II)[2] prompted us to synthesize a series of 3-arylpyridazino[4,3-*c*]isoquinolines (III) and evaluate their binding properties to this receptor (Chart I). On the basis of the structural similarity of the three classes of compounds it could reasonably be expected that they would interact with the BZ receptors in a similar way. Thus, a limited number of compounds belonging to class III were prepared, and their ability to displace [^{3}H]diazepam (DZ) was assessed. Results of this study indicated that the enlargement of ring C from pyrazolo or triazolo to pyridazine did not greatly affect the binding. A series of derivatives of class III was then synthesized in order to clarify the influence of the substituents in positions 3 and 6 on the biological activity.

Chemistry. We previously reported[4] that the hydrazones obtained by condensation of *N*-aminophthalimidine with ethyl benzoylacetate or acetoacetate undergo a sodium ethoxide promoted rearrangement to give 4(1*H*)-pyridazones **1a** or **1d** (Scheme I) as the main products. This procedure was also employed in the conversion of the hydrazones prepared from 4-methoxy- and 4-chloro-substituted benzoylacetates[5] to yield **1b** and **1c**, respectively.

Lactonization of **1a–d** was achieved either with an equimolar amount of dicyclohexylcarbodiimide in refluxing pyridine[4] or with acetic anhydride in toluene with the azeotropic removal of acetic acid.

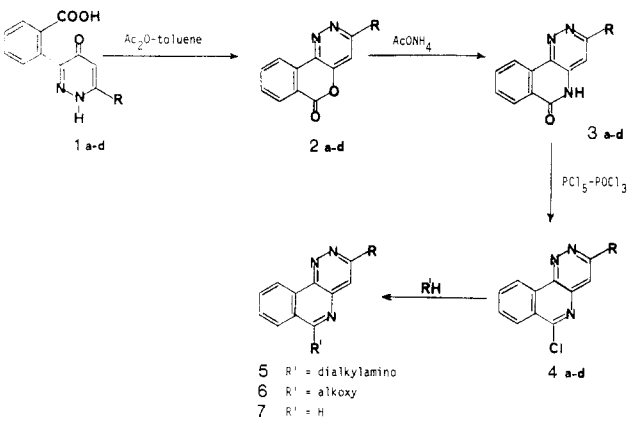
Treatment of **2a–d** with an excess of dry ammonium acetate at ca. 200 °C in a steel cylinder quantitatively gave lactams **3a–d**, which were then treated with phosphorus pentachloride and phosphorus oxychloride to yield **4a–d**. The physical properties of intermediates **1–4** are shown in Table I. Substitution of the chlorine atom of **4a–d** with various dialkylamines gave 6-(dialkylamino)pyridazino-[4,3-*c*]isoquinolines **5** while 6-alkoxypyridazino[4,3-*c*]isoquinolines **6** were obtained by reaction of **4a–d** with sodium alkoxides in the corresponding alcohol. Hydrogenation of **4a**, in the presence of palladium on carbon with magnesium oxide as an acid acceptor, caused displacement of the chlorine atom and saturation of the 5,6 double bond. Oxidation of the dihydro derivative to **7** was achieved by treatment with an ethanolic solution of iodine and po-

Chart I. 1,2,4-Triazolo[3,4-*a*]phthalazines (I), Pyrazolo[4,3-*c*]quinolin-3(5*H*)-ones (II), and Pyridazino[4,3-*c*]isoquinolines (III)[a]



[a] Key: Ar = substituted phenyl; R = dialkylamino, alkoxy, hydrogen.

Scheme I[a]



[a] For compounds **1–4**: a, R = C_6H_5; b, R = 4-$OCH_3C_6H_4$; c, R = 4-ClC_6H_4; d, R = CH_3.

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(2) The pyrazoloquinolines CGS 9896 and CGS 8216, which differ in a chlorine atom, are respectively an agonist and antagonist at the BZ receptor as reported by: Yokoyama, N.; Ritter, B.; Neubert, A. D. *J. Med. Chem.* **1982**, *25*, 337.

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Table I. Intermediates 1-4 of Scheme I

no.	yield,[a] %	mp, °C	cryst solvent	formula[b]	^{1}H NMR spectral data[c] s, 1, H_5	br, 1, NH
1a	77	214–216 dec	EtOH	$C_{17}H_{12}N_2O_3$	6.62	13.2–15.0
1b	74	224–227 dec	MeOH	$C_{18}H_{14}N_2O_4$	6.60	13.47 (s)
1c	79	270–272 dec	MeOH	$C_{17}H_{13}ClN_2O_4$[d]	6.72	13.8–14.2
1d	66	209–210 dec	EtOH	$C_{12}H_{10}N_2O_3$	6.22	12.4–13.6

no.	yield,[a] %	mp, °C	cryst solvent	formula[b]	^{1}H NMR spectral data[c] s, 1, H_4	dd, 1, H_{10}	s, 1, NH
2a	92	205–206	AcOEt	$C_{17}H_{10}N_2O_2$	7.65*	8.94 (J = 8.5, 1.5)	
2b	84	230–231	AcOH	$C_{18}H_{12}N_2O_3$	8.32	8.88 (J = 9, 1.5)	
2c	83	273–275	AcOH	$C_{17}H_9ClN_2O_2$	8.49	9.03 (J = 8.5, 1.5)	
2d	97	218–219	AcOEt	$C_{12}H_8N_2O_2$	7.35*	8.95 (J = 8, 1.5)	
3a	97	340–342	EtOH	$C_{17}H_{11}N_3O$	7.65	8.87 (J = 8.5, 2)	11.87
3b	94	314–315	AcOH	$C_{18}H_{13}N_3O_2$	7.80	9.07 (J = 9, 1.5)	12.20
3c	71	>350	AcOH	$C_{17}H_{10}ClN_3O$	7.82	9.04 (J = 9, 1.5)	12.27
3d	96	>350	DMF	$C_{12}H_9N_3O$	7.88	8.76 (J = 8, 1.5)	13.18
4a	94	177–178	Me_2CO	$C_{17}H_{10}ClN_3$[e]	8.37*	9.53 (J = 9, 1.5)	
4b	98	224–227	MeC_6H_5	$C_{18}H_{12}ClN_3O$[f]	8.83	9.51 (J = 8.5, 1.5)	
4c	98	228–230	MeC_6H_5	$C_{17}H_9Cl_2N_3$	8.94	9.53 (J = 8.5, 1.5)	
4d	89	168–169	Me_2CO	$C_{12}H_8ClN_3$	7.86*	9.50 (J = 9, 1.5)	

[a] The yield is based on recrystallized compounds for 1 and 4 and on crude reaction products for 2 and 3. See Experimental Section. [b] The compounds were analyzed for C, H, and N; analytical results were within ±0.4% of theoretical values except for **4a** and **4b**. [c] Chemical shifts in δ for the indicated protons in Me_2SO-d_6 (* in $CDCl_3$); coupling constants, J ortho and meta, in Hz. [d] Obtained as monohydrate; desolvation at 130–150 °C and decomposition at 272 °C as shown by DSC. [e] C: calcd, 69.99; found, 69.44. [f] C: calcd, 67.19; found, 67.70.

tassium acetate. The physical properties of pyridazino[4,3-c]isoquinolines **5–7** are listed in Table II.

Biological Results and Discussion. The in vitro activity in the BZ binding assay of 3,6-disubstituted pyridazino[4,3-c]isoquinolines is shown in Table II. By keeping the phenyl ring constant in position 3 we determined the effect of the 6-substituent of this series in inhibiting the specific [^{3}H]DZ binding. In contrast to the unsubstituted compound **7**, which has a very low affinity for this receptor, it appeared that compounds possessing dialkylamino groups (**5a–e**) show strongly enhanced affinity with K_i values lower than those obtained for Medazepam and Cl 218872. Members of this series bearing an alkoxy group (**6a–b**) show an affinity approaching lactam **3a**, whereas the presence of a chlorine atom (**4a**) diminishes affinity as compared to **3a**. Therefore, compounds bearing 6-dialkylamino groups and selected substituents on the phenyl ring were investigated. The *p*-methoxy and -chloro substituents were considered in order to compare the affinities of these compounds with those reported for classes I[3] and II[2] (Chart I). Compounds having the 4-OCH_3 group (**5f–l**) showed a lower affinity than the corresponding compounds with the unsubstituted phenyl, whereas the presence of the chlorine group practically abolished binding affinity in these molecules (**5m–p**). Thus, the substituent in the 3-position also influences the interaction of these compounds with the BZ receptors. The unexpected good affinity of **5q**, which possesses a methyl group at the 3-position, precludes any correlation of structure and activity on the basis of steric or electronic effects.

The influence of the various dialkylamino groups on BZ receptor binding can be ranked in decreasing order: 1-pyrrolidinyl > dimethylamino = azetidinyl > $N(CH_3)C$-$H_2CHOHCH_3$ > 4-morpholinyl > 1-piperidinyl > $N(C$-$H_2CH_2OCH_3)_2$ in the series of 3-aryl-substituted compounds. To ascertain whether members of this series with K_i's <1500 nM act as agonists or antagonists, we determined displacement curves of [^{3}H]flunitrazepam in the presence and in the absence of GABA (GABA ratio). The majority of compounds acted as partial agonists (GABA ratio 1.2–1.7) like Cl 218872; **5h** acted as a full agonist like Medazepam, whereas **5q** acted like Ro 15-1788, a reference antagonist.[6] The indirect Hill coefficient for all compounds ranges from 0.7 to 0.8, which is indicative of apparent heterogeneity of binding sites or negatively cooperative interactions.[7] Finally, we carried out saturation studies in the presence and in the absence of compounds **5d** and **5q** and applied the Scatchard analysis[8] to the data. This analysis shows whether the inhibition of [^{3}H]DZ binding is due to the occupation by the test compounds of the binding sites or if it is due to a decreased affinity of [^{3}H]DZ for BZ receptors. As shown in Figure 1, the antagonism is competitive since the maximum number of binding sites is unaffected by the presence of **5d** and **5q**, whereas the affinity of [^{3}H]DZ for BZ receptors is reduced. The evaluation of the pharmacological properties of 3,6-disubstituted pyridazino[4,3-c]isoquinolines was limited to compounds with K_i <1500 nM. The anticonflict effect

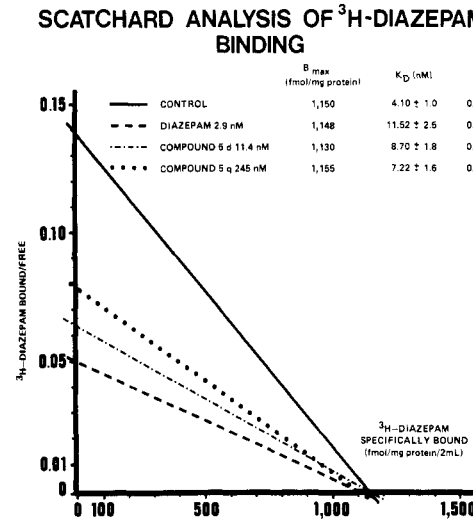

Figure 1. Regression lines as the mean of three experiments each done in triplicate. See the Experimental Section. Key: B_{max} = maximum number of specific binding sites; K_D = dissociation constant; r = correlation coefficient.

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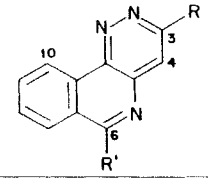
Table II. 3,6-Disubstituted Pyridazino[4,3-c]isoquinolines



no.	R	R′	yield, %	mp, °C	cryst solvent	formula[a]	^{1}H NMR spectral data[b] s, 1, H_4	^{1}H NMR spectral data[b] dd, 1, H_{10}	BZ-receptor binding in vitro K_i, nM ±SE	BZ-receptor binding in vitro GABA ratio[d]
5a	C_6H_5	$N(CH_3)_2$	81	135–137	Me_2CO	$C_{19}H_{16}N_4$	7.99	9.36 (J = 9, 1.5)	28.4 ± 1.2	1.2
5b	C_6H_5	$N(CH_3)C_2H_5$	71	130–133	EtOAc	$C_{20}H_{18}N_4$	8.03	9.39 (J = 8, 2)	65.2 ± 3.0	1.5
5c	C_6H_5	$N(CH_3)CH_2CHOHCH_3$	77	161–162	EtOH	$C_{21}H_{20}N_4O$	7.90	9.31 (J = 8.5, 1.5)	136 ± 7.6	1.4
5d	C_6H_5		85	174–176	EtOAc	$C_{21}H_{18}N_4$	7.80	9.33 (J = 8, 2.5)	11.4 ± 0.6	1.6
5e	C_6H_5		82	214–216	EtOAc	$C_{21}H_{18}N_4O$	8.03	9.37 (J = 8.5, 2.5)	152 ± 8.9	1.4
5f	4-$OCH_3C_6H_4$	$N(CH_3)_2$	45	160–162	Me_2CO	$C_{20}H_{18}N_4O$	8.00	9.42 (J = 8.5, 1.5)	63.3 ± 3.4	1.7
5g	4-$OCH_3C_6H_4$	$N(CH_3)CH_2CHOHCH_3$	45	147–149	Me_2CO	$C_{22}H_{22}N_4O_2$	7.91	9.40 (J = 9, 1.5)	221 ± 12	1.4
5h	4-$OCH_3C_6H_4$	$N(CH_2CH_2OCH_3)_2$	94	127–128	MeOH	$C_{24}H_{26}N_4O_3$	8.05	9.48 (J = 9, 1.5)	1180 ± 68	2.0
5i	4-$OCH_3C_6H_4$		93	196–198	C_6H_6	$C_{21}H_{18}N_4O$	7.43	9.40 (J = 9, 1.5)	69.6 ± 3.8	*e*
5j	4-$OCH_3C_6H_4$		89	176–177	EtOAc	$C_{22}H_{20}N_4O$	7.89	9.44 (J = 9, 1.5)	18.7 ± 1.6	*e*
5k	4-$OCH_3C_6H_4$		88	168–169	EtOAc	$C_{23}H_{22}N_4O$	8.10	9.44 (J = 8.5, 1.5)	658 ± 40	*e*
5l	4-$OCH_3C_6H_4$		85	218–220	C_6H_6	$C_{22}H_{20}N_4O_2$	8.11	9.47 (J = 9, 1.5)	402 ± 24	*e*
5m	4-ClC_6H_4	$N(CH_3)_2$	78	175–177	EtOAc	$C_{19}H_{15}ClN_4$	7.95	9.35 (J = 8.5, 1.5)	9300 ± 395	*f*
5n	4-ClC_6H_4	$N(CH_3)CH_2CHOHCH_3$	74	151–153	Me_2CO	$C_{21}H_{19}ClN_4O$	8.18*	9.27 (J = 9, 1.5)	17800 ± 1107	*f*
5o	4-ClC_6H_4	$N(CH_2CH_2OCH_3)_2$	80	123–125	MeOH	$C_{23}H_{23}ClN_4O_2$	8.07	9.47 (J = 8.5, 1.5)	32500 ± 1890	*f*
5p	4-ClC_6H_4		55	212–214	EtOAc	$C_{21}H_{17}ClN_4$	7.89	9.44 (J = 8.5, 1.5)	3480 ± 138	*f*
5q	CH_3		55	150–152	EtOAc	$C_{16}H_{16}N_4$	7.42	9.43 (J = 8.5, 1.5)	245 ± 98	1.0
6a	C_6H_5	OC_2H_5	92	155–156	Me_2CO	$C_{19}H_{15}N_3O$[g]	8.02	9.28 (J = 8.5, 1.5)	1500 ± 103	*f*
6b	C_6H_5	$OCH(CH_3)_2$	95	132–133	Me_2CO	$C_{20}H_{17}N_3O$	8.12	9.37 (J = 8.5, 1.5)	1900 ± 99	*f*
7	C_6H_5	H	76	182–183	Me_2CO	$C_{17}H_{11}N_3$	8.43	9.42 (J = 8.5, 1.5)	4000 ± 250	*f*
4a	C_6H_5	Cl	*h*						6200 ± 333	*f*
3a	C_6H_5	OH							1700 ± 80	*f*
diazepam									2.90 ± 0.20	2.3
medazepam									528 ± 20	1.9
Cl 218872									198 ± 10	1.2
Ro 15-1788									1.94 ± 0.15	0.8

[a] The compounds were analyzed for C, H, and N; analytical results were within ±0.4% of theoretical values except for **6a**. [b] Chemical shifts in δ for the indicated protons in $CDCl_3$ (* in Me_2SO-d_6); coupling constants, J ortho and meta, in Hz. [c] $K_i = IC_{50}/(1 + C/K_D)$ where C = concentration of free [^{3}H]diazepam and K_D = apparent dissociation constant. IC_{50} values were assessed from at least six concentrations in triplicate, and the determinations were repeated at least twice. [d] IC_{50} compound/IC_{50} compound + GABA 10^{-4} M. [e] Not determined because the displacement curves in the presence and in the absence of GABA were not parallel. [f] Test not done because $K_i \gg 1000$. [g] C: calcd, 75.73; found, 76.29 [h] See Table I.

Table III. Biological Activities of Selected Compounds

no.	LD_{50} (mice), mg/kg ip	Vogel test (rats) MED,[a] mg/kg ip	anticonvulsant act. (mice), ED_{50},[b] mg/kg ip	
			Metrazol	Bicuculline
5a	>600	10	>100	>100
5b	300	10	>50	>50
5c	300	30	>50	>50
5d	300	5	>50	>50
5e	200	15	>50	>50
5f	>600	10	>100	>100
5g	>600	30	>100	>100
5h	>600	10	>100	>100
5i	600	30	>100	>100
5j	>600	20	>100	>100
5k	>600	10	>100	>100
5l	>600	30	>100	>100
5q	200	20	>50	>50
diazepam		0.5	0.2 (0.11–0.28)	0.18 (0.13–0.25)

[a] Minimal effective dose that significantly (Mann-Whitney U-test) increased the number of shocks in comparison with controls; ten animals per dose used. [b] Dose that prevented tonic extensor seizures in 50% of the animals; 95% confidence limits in parentheses; ten animals per dose used.

was assessed in rats by the Vogel procedure, and the anticonvulsant activity was determined in mice after metrazol or bicuculline challenge. The test compounds were administered ip, and the results are shown in Table III together with LD_{50} values.

A substantial anticonflict effect (MED 5 mg/kg ip) was elicited by **5d**, which also exhibited the highest affinity for BZ receptors (K_i = 11.4 nM). However **5h** (K_i = 1180 nM) was only 2 times less active than **5d**, whereas **5j** (K_i = 18.7 nM) was 4 times less active than **5d**. It is worth noting that all compounds showed anticonflict effects over a narrow range of doses (MED 5–30 mg/kg ip). However, none prevented convulsions induced by either metrazol or bicuculline at doses up to 50 mg/kg ip (when their LD_{50} values were ≤300 mg/kg ip) or up to 100 mg/kg ip (when LD_{50} values were ≥600 mg/kg ip). This dissociation between anticonflict and anticonvulsant activity found in compounds with affinity for BZ receptors is remarkable[9] and is considered to be an indication of selective anxiolytics.[1] In order to better evaluate the advantages of **5d**, we studied its ataxic side effects by means of the rotarod test. No neuromuscular impairment was observed up to the highest dose tested, 50 mg/kg ip, in rats.

Finally, the weak anticonflict activity (MED 20 mg/kg ip) of compound **5q**, the only one of the series bearing a 3-methyl group, was unexpected in a compound with GABA ratio = 1. Therefore, we studied **5q** as an antagonist to the muscle relaxant action of DZ by means of the traction test in mice. Indeed, the muscle relaxation caused by 3 mg/kg ip of DZ was antagonized in five out of 10 animals by 20 mg/kg ip of **5q** whereas 10 mg/kg ip was ineffective.

Conclusions. A series of 3-aryl- (or 3-methyl-) 6-(dialkylamino)pyridazino[4,3-c]isoquinolines (**5a–l** and **5q**) displace [^{3}H]DZ from cerebral receptor sites with different potencies. Like BZs, they increase punished responses in the rat conflict procedure, but unlike BZs they lack activity in anticonvulsant tests. 3-Phenyl-6-pyrrolidinylpyridazino[4,3-c]isoquinoline (**5d**) shows the highest anticonflict activity (MED 5 mg/kg ip), does not produce neuromuscular impairment up to 50 mg/kg ip, and has a good therapeutic ratio (LD_{50} = 300 mg/kg ip). Thus, **5d** appears to be a novel and selective anxiolytic agent in animal models.

(9) This dissociation has been observed in two quinoline derivatives PK 8165 and PK 9084 as reported by: Le Fur, G.; Mizoule, J.; Burgevin, M. C.; Ferris, O.; Heaulme, M.; Gauthier, A.; Gueremy, C.; Uzan, A. *Life Sci.* **1981**, *28*, 1439.

Experimental Section

Melting points were determined on a Büchi SMP-510 capillary apparatus and are uncorrected. Differential scanning calorimetry (DSC) curves were obtained on a TA 2000 Mettler thermal analyzer, in a normal pan, with a heating rate of 5 °C/min. IR (Perkin-Elmer 157) and ^{1}H NMR spectra (Brüker WP 60 or WH 270 MHz) were obtained for all compounds and were consistent with the assigned structures. The elemental analyses were performed by the Analytical Department of Gruppo Lepetit. TLC was performed on Merck silica gel plates 60F-254, visualized with UV light and/or I_2 vapors.

2-(1,4-Dihydro-4-oxo-3-pyridazinyl)benzoic Acids (1a–d). The hydrazones, obtained according to the published method[10] by condensation of *N*-aminophthalimidine with substituted benzoylacetates or acetoacetate, were used without purification in the conversion to **1a–d** using sodium ethoxide in absolute ethanol. The procedure described[4] for the preparation of **1a** was representative of all cases.

3-Phenyl-6*H*-[2]benzopyrano[4,3-*c*]pyridazin-6-one (2a). A mixture of 10 g (0.034 mol) of **1a** in 100 mL of toluene and 100 mL of acetic anhydride was stirred and heated in a flask equipped with a distillation column, and the fraction boiling between 96 and 108 °C (~100 mL) was collected at atmospheric pressure in 1.5 h. The solid gradually dissolved during this distillation, and the resulting solution was subsequently evaporated under reduced pressure. The residue was taken up with 250 mL of methylene chloride, and the resulting solution was washed with 5% sodium bicarbonate and then with water and dried ($MgSO_4$). Evaporation of the solvent gave 9.2 g (92%) of crude **2a**, which was sufficiently pure for use in the next step (TLC: C_6H_6–EtOAc, 8:2). An analytical sample was obtained after recrystallization from ethyl acetate: mp 205–206 °C; IR (Nujol) ν_{max} 1760, 1620, 1600, 775, 745, 690 cm^{-1}.

Lactone **2d** was prepared as described above for **2a**. In the cases of **2b** and **2c**, the distillation of the low-boiling fraction required 3 h and the residues from the evaporation of toluene–acetic anhydride were triturated with toluene, collected by filtration, and used as such. The analytical samples were obtained after recrystallization from acetic acid.

3-Arylpyridazino[4,3-*c*]isoquinolin-6(5*H*)-ones (3a–d). General Procedure. A mixture of 9 g (0.033 mol) of **2a** and 90 g of dry ammonium acetate was heated at 190–200 °C for 9 h in a steel cylinder. The cooled reaction mixture was triturated with water and filtered to give 8.7 g (97%) of crude **3a** which was sufficiently pure for use in the next step (TLC: CH_3OH–$CHCl_3$, 1:9). An analytical sample was obtained after recrystallization from ethanol: mp 340–342 °C; IR (Nujol) ν_{max} 1660, 1600, 1550, 1340, 685 cm^{-1}.

The same procedure was employed for the preparation of **3b–d**.

6-Chloro-3-phenylpyridazino[4,3-*c*]isoquinoline (4a). A mixture of 7.4 g (0.027 mol) of **3a** and 5.83 g (0.028 mol) of phosphorus pentachloride in 160 mL of phosphorus oxychloride was stirred and heated at reflux for 3.5 h. The solid gradually dissolved, and the resulting solution was evaporated under reduced pressure. The residue was triturated with 10% ammonium acetate, collected by filtration, and recrystallized from acetone to give 7.4 g (94%) of **4a**: mp 177–178 °C; IR (Nujol) ν_{max} 1570, 1480, 960, 760, 685 cm^{-1}. Anal. ($C_{17}H_{10}ClN_3$) N, H; C: calcd, 69.99; found, 69.44.

In the preparations of **4b** and **4c**, equimolar amounts of dry pyridine were added to the reaction mixtures, which were then heated at reflux for 4.5 h. In the absence of pyridine too great an excess of phosphorus oxychloride would have had to be used in order to obtain a solution. In the preparation of **4d**, it was necessary to prolong the reflux time to 7 h to complete the re-

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action. Anal. ($C_{18}H_{12}ClN_3O$ (**4b**))N, H; C: calcd. 67.19; found, 67.70.

6-(Dialkylamino)pyridazino[4,3-*c*]isoquinolines 5a–q. General Procedure. A mixture of 0.02 mol of **4** and 0.044 mol of the appropriate dialkylamine in 100 mL of 1,2-dimethoxyethane (**4a**, **4d**) or diethylene glycol dimethyl ether (**4b**, **4c**) was stirred and heated at reflux for 2 h. Reactions were run in a steel cylinder heated at 120–140 °C for 8 h, using volatile dialkylamines. All dialkylamines were commercially available except 1-(methylamino)-2-propanol used in the synthesis of **5c,g,n**, which was prepared from methylamine and propylene oxide.[11] In all cases, the solvent was then removed under reduced pressure, the residue triturated with 200 mL of water, and the solids collected via filtration. The crude products were recrystallized from the solvents listed in Table II.

6-Alkoxypyridazino[4,3-*c*]isoquinolines (6a,b). General Procedure. To a solution of 0.5 g (0.021 mol) of sodium in 250 mL of the appropriate anhydrous alcohol was added 5.83 g (0.02 mol) of **4a** in portions, and the reaction mixture was stirred at 60 °C for 1.5 h. The solvent was evaporated under reduced pressure, and the residue was triturated with water. The insoluble material was collected via filtration and recrystallized from the solvents listed in Table II. Anal. ($C_{19}H_{15}N_3O$ (**6a**) N, H; C: calcd, 75.73; found, 76.29.

3-Phenylpyridazino[4,3-*c*]isoquinoline (7). A solution of 12.5 g (0.043 mol) of **4a** in 1.5 L of 2-methoxyethanol was hydrogenated at room temperature and atmospheric pressure in the presence of 2.5 g of 10% palladium on carbon and 1.8 g (0.044 mol) of magnesium oxide. After about 1700 mL of hydrogen was absorbed, the mixture was filtered, the solvent removed from the filtrate under reduced pressure, and the residue recrystallized from 2-propanol to give 15 g (74%) of 5,6-dihydro-3-phenylpyridazino[4,3-*c*]isoquinoline: mp 250–252 °C; IR (Nujol) ν_{max} 1610, 1560, 1410, 1345, 770 cm^{-1}; NMR ($CDCl_3$) δ 4.72 (s, 2, CH_2), 7.07 (s, 1, H_4), 7.20 (br, 1, NH), 6.8–8.2 (m, 8, aromatic), 8.4 (dd, J = 8.5 and 1.5 Hz, 1, H_{10}). Anal. ($C_{17}H_{13}N_3$) N, H; C: calcd, 78.74; found, 78.27. To a boiling solution of 3.9 g (0.015 mol) of this dihydro derivative and 14.7 g (0.15 mol) of potassium acetate in 600 mL of ethanol was added dropwise a solution of 3.8 g (0.015 mol) of iodine in 150 mL of ethanol. The reaction mixture was heated at reflux for an additional 2 h, and the solvent was then evaporated under reduced pressure. The residue was triturated with water and the insoluble material was collected via filtration. This crude product was chromatographed on a silica gel column eluted with 1% CH_3OH in $CHCl_3$ to give 2.95 g (76%) of **7**: mp 182–183 °C; IR (Nujol) ν_{max} 1600, 1580, 1500, 765, 690 cm^{-1}; NMR ($CDCl_3$) δ 7.5–8.4 (m, 8, aromatic), 8.43 (s, 1, H_4), 9.40 (s, 1, H_6), 9.42 (dd, J = 8.5 and 1.5 Hz, 1, H_{10}).

Biological Test Procedures. Diazepam (DZ) and Medazepam were purchased from FIS,Ro 15-1788 was obtained from Dr. W. Haefely (Hoffmann-La Roche-Basle), and Cl 218872 was synthesized in our laboratories following the patented procedure.[12] [^{3}H]DZ with specific activity 87.5 Ci/mmol and [^{3}H]flunitrazepam with specific activity 72.4 Ci/mmol were purchased from New England Nuclear, Boston, MA. The radioactivity was measured in a 460 C Packard liquid scintillation spectrometer. The homogenate was obtained with a Brinkman-Polytron PT 10 microhomogenizer, setting 7 for 20 s.

Benzodiazepine-Receptor Binding in Vitro. [^{3}H]DZ binding studies were carried out according to the method of Möhler and Okada,[13] incubating [^{3}H]DZ (0.65–1.20 nM) with rat forebrain synaptosomes. Specific binding was determined by subtracting the binding in the presence of 3 μM cold DZ from the binding in the presence of [^{3}H]DZ alone (total binding − nonspecific binding). The concentrations of the test compounds that cause 50% inhibition of the specific [^{3}H]DZ binding (IC_{50}) were assessed from at least six concentrations in triplicate. All determinations of IC_{50} were repeated at least twice. The inhibition curves were transformed into straight lines according to log-probit analysis.[14] In saturation studies, 10 different [^{3}H]DZ concentrations from 0.05 to 40 nM were incubated in triplicate with the compounds under evaluation at the respective K_i concentrations or without them (controls). The nonspecific binding was determined in triplicate for each concentration of [^{3}H]DZ. The different regression lines were compared for the significance of differences ($p < 0.01$) in slopes and intercepts by the method of Colton.[15]

The GABA ratio was determined according to the method of Wastek et al.[16] in the rat forebrain. One milliliter of membranes was incubated in triplicate with 0.4 nM [^{3}H]flunitrazepam and various concentrations of the ligand, in the presence or absence of 0.1 mM GABA for 20 min at 37 °C. The binding in the presence of 1 μM cold Clonazepam was subtracted from the binding in the absence of excess Clonazepam to obtain the specific binding. IC_{50} values were assessed as the concentration of test compound that caused 50% inhibition of specific [^{3}H]flunitrazepam binding. Student's t-test was used to evaluate the statistical significance of differences between IC_{50} values. The indirect Hill coefficient for each compound was determined by Hill plot analysis[7] of the inhibition curve of [^{3}H]flunitrazepam. The in vitro binding data were calculated on an Apple II microcomputer with the Recept Program described by Benfenati and Guardabasso.[17]

Vogel Conflict Procedure. The Vogel procedure in unconditioned rats as modified by Lippa et al.[18] was used. Male Wistar rats deprived of food (24 h) and water (48 h) were placed in a black Plexiglass test chamber. A sweetened milk solution was available through a stainless-steel tube placed on the back wall. The rats were allowed 15 s of free drinking; after that, an electric shock (0.3 mA) was applied through the drinking tube in alternating 5-s on–off shock cycles for a total of 5 min. The number of shocks received was recorded. Test compounds dispersed in 0.5% methocel at a volume of 4 mL/kg were given ip to 10 rats/dose 30 min before the experiment while the control groups were treated with the vehicle. The minimal effective dose (MED), i.e. the dose that significantly increased the number of shocks in comparison with controls, was determined. The significance was assessed by the Mann–Whitney U-test.[19]

Metrazol Anticonvulsant Test. The method described by Berger[20] was employed. Test compounds dispersed in 0.5% methocel at a volume of 10 mL/kg were given ip to 10 male CD_1 mice per dose and 30 min later 140 mg/kg of an aqueous solution of metrazol was administered subcutaneously. The control groups treated with the vehicle and metrazol developed convulsions and died within 30 min. The number of survivors at 2 h in the experimental group was recorded. ED_{50} was calculated by the probit analysis of Finney[21] as the dose that prevented tonic extensor seizures in 50% of the mice.

Bicuculline Anticonvulsant Test. The method described by De la Mora[22] was employed. An aqueous solution of bicuculline was administered subcutaneously at the dose of 2 mg/kg 30 min after the treatment with test compounds following the experimental procedure described for the metrazol test. Male CD_1 mice were used.

Traction Test. The muscular relaxation was evaluated according to the method of Julou-Courvoisier as described by Boissier et al.[23] The apparatus used consisted of a metal rod 2.5

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mm in diameter and 30 cm in length fixed horizontally 15 cm above the platform. Male CD_1 mice were hung from the rod by their forepaws. Normal animals climb on the rod within 4 s, hanging by all four paws, whereas animals with impairment of muscular tone fall from the rod or continue to hang by the forepaws only. At the dose of 3 mg/kg ip, DZ caused muscle relaxation in nearly all the animals 30 min after treatment. Test compounds dispersed in 0.5% methocel at a volume of 10 mL/kg were administered at 10 and 20 mg/kg ip 15 min after DZ to 10 mice at each dose. Fifteen minutes later, the mice were suspended by means of their forepaws to the rod and the percentage of them falling from it was recorded.

Rotarod Test. The effect on motor coordination was determined by the method of Dunham and Miya[24] in male Wistar rats. The rod was 6 cm in diameter and 56 cm in length, fixed horizontally 15 cm above the support and was rotated at a speed of 6 rpm. The control groups treated with the solvent alone remained on the rod for at least 5 min. Ten animals per dose were placed on the rod 30 and 60 min after treatment with test compounds dispersed in 0.5% methocel at a volume of 4 mL/kg. The animals that fell off the rod during the 5-min session were recorded.

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Acute Toxicity. Test compounds were dispersed in 0.5% methocel at a volume of 10 mL/kg and administered ip to CD_1 male mice arranged in groups of three for each dose, i.e. 600–300–100 mg/kg. The animals were observed for 1–5 days, and LD_{50} values were graphically calculated.

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Registry No. **1a**, 73351-33-4; **1b**, 96825-70-6; **1c**, 96825-71-7; **1d**, 73351-34-5; **2a**, 73351-35-6; **2b**, 96825-72-8; **2c**, 96825-73-9; **2d**, 73351-36-7; **3a**, 96825-74-0; **3b**, 96825-75-1; **3c**, 96825-76-2; **3d**, 96825-77-3; **4a**, 96825-78-4; **4b**, 96825-79-5; **4c**, 96825-80-8; **4d**, 96825-81-9; **5a**, 96825-82-0; **5b**, 96825-83-1; **5c**, 96825-84-2; **5d**, 96826-01-6; **5e**, 96825-85-3; **5f**, 96825-86-4; **5g**, 96825-87-5; **5h**, 96825-88-6; **5i**, 96825-89-7; **5j**, 96825-90-0; **5k**, 96825-91-1; **5l**, 96825-92-2; **5m**, 96825-93-3; **5n**, 96825-94-4; **5o**, 96825-95-5; **5p**, 96825-96-6; **5q**, 96825-97-7; **6a**, 96825-98-8; **6b**, 96825-99-9; **7**, 96826-00-5; dimethylamine, 124-40-3; *N*-methylethylamine, 624-78-2; *N*-methyl(2-hydroxypropyl)amine, 16667-45-1; pyrrolidine, 123-75-1; morpholine, 110-91-8; azetidine, 503-29-7; piperidine, 110-89-4.

Synthesis of High Specific Activity [^{75}Br]- and [^{77}Br]Bromperidol and Tissue Distribution Studies in the Rat

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A rapid synthesis of [^{75}Br]- and [^{77}Br]bromperidol with specific activity exceeding 10 000 Ci/mmol is described in which a trimethylstannylated analogue of bromperidol is used as a substrate for regiospecific no-carrier-added radiobromination. 4-[4-[4-(Trimethylstannyl)phenyl]-4-hydroxypiperidino]-4′-fluorobutyrophenone was synthesized by the reaction of (trimethylstannyl)sodium with haloperidol and purified by preparative HPLC. Subsequent radiobromination with no-carrier-added $^{75}Br^-$ or $^{77}Br^-$ and in situ oxidation using H_2O_2/CH_3COOH gave a corrected radiochemical yield of 35% with a 30-min preparation time. Tissue distribution studies in the rat show a rapid and prolonged uptake into the brain, liver, and kidneys and consistently low blood concentrations that differ quantitatively from previous studies using relatively low specific activity bromperidol. Potential clinical applications for this high specific activity radiobrominated neuroleptic are discussed.

Pharmacokinetic data for neuroleptics of the butyrophenone class are scarce.[1-3] The conventional approach to assessing butyrophenone pharmacokinetic parameters in man is to measure serum concentrations of the neuroleptic using gas–liquid chromatographic[4-8] or high-performance liquid chromatographic[9,10] methods, but these techniques unfortunately have low sensitivity (0.5–1.0 and 2–3 ng/mL, respectively). While radioimmunoassay has been suggested as an alternative analytical method,[11,12] it has an even lower sensitivity of 3–10 ng/mL[13] and has shown poor cross-correlation.[14]

The wide variation in clinical responses reported for neuroleptic serum concentrations[15] may indicate the error in assuming that the brain concentration and pharmacological activity of the butyrophenones are proportional to their blood concentration. In early reports concerning butyrophenone neuroleptics,[16] it was suggested that the

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