113508-31-9; **82** (ethyl ester), 113508-47-7; **83**, 113508-32-0; **83** (ethyl ester), 113508-48-8; **84**, 113508-60-4; **85**, 113508-33-1; **86**, 113508-61-5; **87**, 113508-34-2; **88**, 113508-35-3; **88** (ethyl ester), 113508-62-6; **89**, 113508-36-4; **90**, 113508-37-5; **90** (ethyl ester), 113508-49-9; **91**, 76577-59-8; **92**, 76577-60-1; **92** (oxime), 76577-61-2; **93**, 76577-63-4; **94**, 76577-88-3; **95**, 113508-38-6; **95**·HCl, 76577-64-5; **96**, 76577-87-2; **97**, 68050-32-8; **97** (ethyl ester), 68050-31-7; **98**, 113508-39-7; **98** (ethyl ester), 113508-50-2; **99**, 68050-36-2; **99** (ethyl ester), 68050-35-1; **100**, 68050-29-3; **101**, 76577-54-3; **102**, 76577-55-4; **102** (oxime), 76577-56-5; **103**, 76577-58-7; **104**, 113508-40-0; **104** (ethyl ester), 113508-51-3; **105**, 68050-25-9; **105** (ethyl ester), 68050-24-8; **106**, 68050-16-8; **106** (ethyl ester), 68050-15-7; **107**, 68050-19-1; **107** (ethyl ester), 68050-18-0; **108**, 113508-41-1; **108** (ethyl ester), 113508-52-4; **109**, 68050-40-8; **109** (ethyl ester), 68050-39-5; **110**, 68050-30-6; **110** (ethyl ester), 68050-29-3; **111**, 68050-21-5; **112**, 68050-46-4; **112** (ethyl ester), 68050-21-5; **113**, 113508-42-2; **113** (ethyl ester), 113508-53-5; **114**, 76002-75-0; **114** (ethyl ester), 76013-27-9; **115**, 76002-76-1; **116**, 76002-77-2; **117**, 76002-86-3; **118**, 76577-47-4; **119**, 76577-41-8; **120**, 76577-43-0; **121**, 76577-42-9; **122**, 76577-46-3; **123**, 76002-83-0; **124**, 76325-62-7; **125**, 76325-51-4; **126**, 113508-65-9; **127**, 76325-47-8; **128**, 76325-49-0; **129**, 76325-50-3; **130**, 76325-52-5; **131**, 76325-54-7; **132**, 76325-56-9; **133**, 76325-55-8; **134**, 76577-90-7; **134**·HCl, 76577-80-5; **135**, 76577-65-6; **136**, 113508-66-0; **137**, 113508-67-1; **138**, 113508-68-2; **140**, 69015-16-3; **142**, 69015-14-1; **143**, 69015-25-4; **144**, 69015-24-3; **145**, 69015-32-3; **146**, 69015-34-5; **147**, 69015-35-6; **147** (ethyl ester), 69040-91-1; **148**, 69015-36-7; **149**, 69015-37-8; **149** (ethyl ester), 69015-26-5; **150**, 69015-33-4; **150** (ethyl ester), 69015-23-2; **151**, 69015-40-3; **151** (ethyl ester), 69015-29-8; **152**, 69015-38-9; **152** (ethyl ester), 69015-27-6; **153**, 69015-39-0; **153** (ethyl ester), 69015-28-7; **154**, 113508-69-3; **154** (ethyl ester), 113508-70-6; **155**, 69015-42-5; **155** (ethyl ester), 69015-31-2; **156**, 69015-54-9; **156** (ethyl ester), 69015-53-8; **157**, 69015-41-4; **157** (ethyl ester), 69015-30-1; **158**, 76577-69-0; **159**, 76577-70-3; **160**, 76577-73-6; **161**, 76577-74-7; **162**, 113508-75-1; **163**, 113532-99-3; **165**, 99867-03-5; **166**, 113508-71-7; **167**, 113508-76-2; **168**, 113508-77-3; **169**, 113508-78-4; **170**, 113508-72-8; **171**, 113508-73-9; **172**, 113508-74-0; **173**, 113508-79-5; **174**, 113508-80-8; **175**, 113508-81-9; **176**, 82296-39-7; **177**, 82296-41-1; **178**, 90510-54-6; **179**, 113508-82-0; **180**, 82296-43-3; **181**, 82296-45-5; **182**, 65565-70-0; **183**, 113508-83-1; **184**, 113508-85-3; **185**, 113508-84-2; **186**, 38922-77-9; **186**·HBr, 2549-17-9; **187**, 76577-82-7; **188**, 76577-83-8; **190**, 77947-41-2; **191**, 88220-27-3; **192**, 60050-77-3; **193**, 113508-91-1; **194**, 113533-00-9; **195**, 113508-86-4; **196**, 113508-92-2; **197**, 113508-87-5; **198**, 64951-05-9; **199**, 64951-09-3; **200**, 81021-97-8; **201**, 113508-88-6; **202**, 113508-93-3; **203**, 113508-89-7; **204**, 113508-94-4; **205**, 113508-90-0; $H_2C{=}CHCN$, 107-13-1; $BrCH_2COCO_2Et$, 10-23-1; $H_3CCHBrCOCO_2Et$, 57332-84-0; $BrCH_2CO_2Et$, 105-36-2; $H_3CC(OEt)_3$, 78-39-7; 5-aminotetrazole, 4418-61-5; 2-amino-4-chloroquinoline, 20151-42-2; 2-amino-4-(methylthioquinoline), 113508-10-4; 2-amino-4-(methylsulfinyl)quinoline, 113508-11-5; ethyl 2-aminoquinoline-3-carboxylate, 36926-83-7; 2-amino-3-(hydroxymethyl)quinoline, 75353-55-8; 2-chloro-7-(trifluoromethyl)quinoline, 83183-56-6; 2-amino-7-(trifluoromethyl)-quinoline, 113508-12-6; 2-chloro-6-nitroaniline, 769-11-9; 2-chloro-6-nitrodihydrocinnamonitrile, 113508-13-7; 2-amino-5-chloroquinoline, 68050-37-3; quinoline, 91-22-5; 2-aminoquinoline, 580-22-3; 2-amino-7-chloroquinoline, 43200-95-9; 2-amino-4,7-dichloroquinoline, 68050-28-2; 2-amino-4-chloro-7-methoxyquinoline, 68050-20-4; glycerolacetonide, 100-79-8; 2-amino-6-chloro-4-phenylquinoline, 51478-40-1; 4,5-dichloro-*o*-phenylenediamine, 5348-42-5; alloxan hydrate, 3237-50-1; dichloroalloxazine, 58590-56-0; 2-amino-6,7-dichloroquinoxaline, 76002-68-1; 2-aminoquinoxaline, 5424-05-5; 2-aminoquinoxaline-3-carboxamide, 67568-30-3; 2-amino-3-chloroquinoxaline, 34117-90-3; 2-amino-3-chloroquinoxalinonium bromide, 76002-73-8; 2,3,6,7-tetrachloroquinoxaline, 25983-14-6; 2-amino-6,7-dichloro-3-ethoxyquinoxaline, 76325-57-0; 2-amino-3-ethylquinazolin-4(3*H*)-one, 2161-26-4; 1-ethylbenzimidazole, 7035-68-9; ethyl propiolate, 623-47-2; 1-methylbenzimidazole, 1632-83-3; 1-butylbenzimidazole, 4886-30-0; quinaldine, 91-63-4; 2-aminobenzothiazole, 136-95-8; 2-amino-6-methoxybenzothiazole, 1747-60-0; 2-amino-5,6-dimethoxybenzothiazole, 29927-08-0; 2-amino-4-methoxybenzothiazole, 5464-79-9.

6-(Alkylamino)-3-aryl-1,2,4-triazolo[3,4-*a*]phthalazines. A New Class of Benzodiazepine Receptor Ligands†

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Some 6-(alkylamino)-3-aryl-1,2,4-triazolo[3,4-*a*]phthalazines have been shown to displace diazepam from rat brain specific binding sites, in vitro, with K_i (nM) values comparable to those of reference benzodiazepines and to have anticonvulsant (pentylenetetrazole test, mice) and anticonflict activity (Vogel test, rat) in vivo. Separation between the doses causing anticonflict effects (Vogel test, rat) and those impairing motor coordination (rotarod test, rat) has been shown for *N*,*N*-bis(2-methoxyethyl)-3-(4-methoxyphenyl)-1,2,4-triazolo[3,4-*a*]phthalazin-6-amine (**80**). This compound, unlike diazepam, was inactive in counteracting the strychnine (mouse) and maximal electroshock (mouse) induced convulsions and in the "aggressive monkey" model. These differences from the classical benzodiazepines in the animal tests indicate that **80** may have some selective anxiolytic activity.

The search for antianxiety agents without nonspecific central nervous system (CNS) depressant side effects has led to the discovery of several classes of compounds chemically unrelated to the benzodiazepines (BZ). The field has been recently reviewed,[1-5] and since then a few more examples of this type of compounds have been reported.[6-10] All these compounds share the property of

† This work is dedicated to Prof. Valdo Mazzi, Institute of Comparative Anatomy, University of Torino (Italy) on occasion of his 70th birthday.

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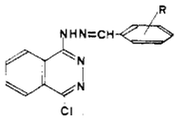
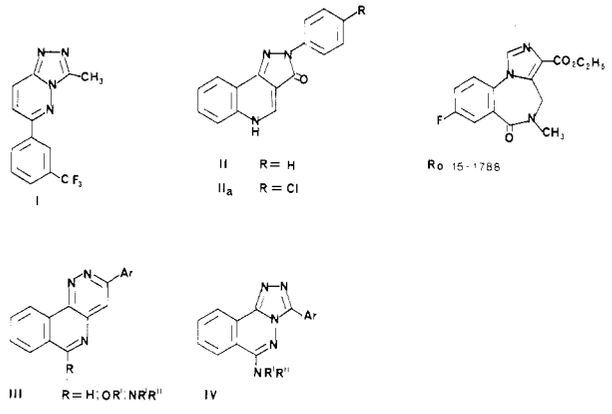
Table I. Araldehydes (4-Chloro-1-phthalazinyl)hydrazones



compd	R	yield, %	mp, °C	solv	formula[a]
1	H	92	174–175	*i*-PrOH	$C_{15}H_{11}ClN_4$
2	4-NHAc	93	222–224	$MeOCH_2CH_2OH$	$C_{17}H_{14}ClN_5O$
3	4-NMe_2	86	173–175	EtOH	$C_{17}H_{16}ClN_5$
4	4-Ph	90	192–194	*i*-PrOH	$C_{21}H_{15}ClN_4$
5	2-Cl, 4-NMe_2	84	233–236	$MeOCH_2CH_2OH$	$C_{17}H_{15}Cl_2N_5$
6	2,4,5-OMe	91	216–218	$MeOCH_2CH_2OH$	$C_{18}H_{17}ClN_4O_3$
7	2-OEt, 3,5-Br	93	184–187	$MeOCH_2CH_2OH$	$C_{17}H_{13}Br_2ClN_4O$
8	2,4,5-OEt	85	170–172	$MeOCH_2CH_2OH$	$C_{21}H_{23}ClN_4O_3$
9	3,4-OMe, 5-Br	96	206–208	EtOH	$C_{17}H_{14}BrClN_4O_2$
10	3-Me, 4-OMe	90	182–185	EtOH–$CHCl_3$	$C_{17}H_{15}ClN_4O$
11	4-Me	81	177–178	EtOH–$CHCl_3$	$C_{16}H_{13}ClN_4$
12	3-Me	93	173–175	EtOH–$CHCl_3$	$C_{16}H_{13}ClN_4$
13	4-OMe	90	175–177	EtOH–$CHCl_3$	$C_{16}H_{13}ClN_4O$
14	4-$CONH_2$	95	285–288	$MeOCH_2CH_2OH$	$C_{16}H_{12}ClN_5O$

[a] All compounds analyzed correctly (±0.3) for C, H, N.

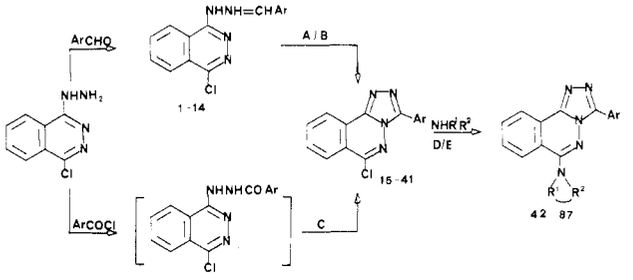
Chart I. Cl-218872 (I), CGS-8216 (II), CGS-9896 (II_a), Pyridazino[4,3-*c*]isoquinolines (III), 1,2,4-Triazolo[3,4-*a*]phthalazine (IV), and Ro 15-1788[a]



[a] Key: Ar = substituted phenyl, R′R″ = dialkyl or cycloalkyl.

displacing [^{3}H]diazepam (^{3}H-DZ) from its specific binding sites in rat brain homogenate in vitro radioligand binding assays, and their pharmacological profiles may range from agonist to antagonist and further to inverse agonist.[5] Rationalization of the structural requirements for the binding of structurally diverse ligands to the BZ receptors has been addressed by several authors.[11] Qualitatively, a frequent feature of these nonclassic BZ ligands is the fusion of a 5- or 6-membered azole ring with another aromatic or heteroaromatic ring system with an approximately planar shape of the resulting molecule. On the basis of this gross simplification and of the structures of 3-methyl-6-[3-(trifluoromethyl)phenyl]-1,2,4-triazolo[4,3-*b*]pyridazine (Cl-218872,[12] I) (Chart I), 2-phenylpyrazolo-[4,3-*c*]quinolin-3(5*H*)-one (CGS-8216,[13] II) (Chart I), and

Scheme I[a]



[a] Key: Ar = substituted phenyl; NR^1R^2 = dialkylamino or cycloalkylamino; A = Br_2/acetic acid/sodium acetate, room temperature 1 h; B = $Pb(OAc)_4$/acetic acid, 45 °C, 1 h; C = ArCOCl/dry dioxane, reflux temperature, 5 h; D = dialkylamine or cycloalkylamine/ethanol, 100 °C, steel bomb, 8 h; E = dialkylamine or cycloalkylamine, 140 °C, steel bomb, 30 h.

2-(4-chlorophenyl)pyrazolo[4,3-*c*]quinolin-3(5*H*)-one (CGS-9896,[14] II_a) (Chart I), we have synthesized a number of 3,6-disubstituted pyridazino[4,3-*c*]isoquinolines[7] (III) (Chart I) and 6-amino-3-aryl-1,2,4-triazolo[3,4-*a*]-phthalazines of general formula IV (Chart I, Scheme I, Table III) that we predicted would bind to ^{3}H-DZ cerebral receptors. We now report our findings for the latter group of compounds, namely their K_i values in the in vitro displacement of ^{3}H-DZ from its specific binding sites of rat brain homogenates and the in vivo anticonflict and anticonvulsant properties of some selected compounds of this series.

Chemistry

The 1,2,4-triazolo[3,4-*a*]phthalazine ring system was first described in 1951 in connection with the study of the antihypertensive activity of hydrazinophthalazine.[15] 4-Chloro-1-phthalazinylhydrazine,[15] prepared from 1,4-dichlorophthalazine, reacts with aromatic aldehydes in diluted hydrochloric acid to yield the corresponding hydra-

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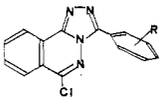
Table II. 3-Aryl-6-chloro-1,2,4-triazolo[3,4-*a*]phthalazines



compd	R	meth (yield, %)	mp, °C	solv	formula[a]
15	H	A (74)	172–174	*i*-PrOH	$C_{15}H_9ClN_4$
16	2-Br	C (60)	203–204	MeOH	$C_{15}H_8BrClN_4$
17	4-CN	C (76)	251–253	$MeOCH_2CH_2OH$	$C_{16}H_8ClN_5$
18	4-NHAc	A (85)	306–308	$MeOCH_2CH_2OH$	$C_{17}H_{12}ClN_5O$
19	4-NMe_2	B (49)	246–248	$MeOCH_2CH_2OH$	$C_{17}H_{14}ClN_5$
20	4-Ph	A (76)	220–222	$MeOCH_2CH_2OH$	$C_{21}H_{13}ClN_4$
21	2-Cl, 4-NMe_2	B (46)	281–283	$MeOCH_2CH_2OH$	$C_{17}H_{13}Cl_2N_5$
22	3,4-Me	C (81)	203–204	*i*-PrOH	$C_{17}H_{13}ClN_4$
23	3-OMe, 4-OC_5H_{11}-*n*	C (82)	150–151	EtOH	$C_{21}H_{21}ClN_4O_2$
24	3,4-OCH_3	C (62)	235–237	$MeOCH_2CH_2OH$	$C_{17}H_{13}ClN_4O_2$
25	3-NO_2, 4-Cl	C (75)	251–253	$MeOCH_2CH_2OH$	$C_{15}H_7Cl_2N_5O_2$
26	2,4,5-OMe	A (73)	189–191	MeOH	$C_{18}H_{15}ClN_4O_3$
27	3,5-Br, 2-OEt	A (63)	184–186	EtOH	$C_{17}H_{11}Br_2ClN_4O$
28	2,4,5-OEt	A (70)	152–153	MeOH	$C_{21}H_{21}ClN_4O_3$
29	3,4-OMe, 5-Br	A (82)	222–224	$MeOCH_2CH_2OH$	$C_{17}H_{12}BrClN_4O_2$
30	3-Cl	C (71)	199–201	EtOH–$CHCl_3$	$C_{15}H_8Cl_2N_4$
31	3-CH_3	A (80)	158–160	EtOH	$C_{16}H_{11}ClN_4$
32	3-CF_3	C (62)	194–196	EtOH–$CHCl_3$	$C_{16}H_8ClF_3N_4$
33	4-Cl	C (76)	166–167	EtOH	$C_{15}H_8Cl_2N_4$
34	4-F	C (65)	174–176	MeOH	$C_{15}H_8ClFN_4$
35	4-Me	A (81)	201–203	EtOH–$CHCl_3$	$C_{16}H_{11}ClN_4$
36	4-OMe	C (80)	190–192	*i*-PrOH	$C_{16}H_{11}ClN_4O$
37	4-O-*i*-Pr	C (70)	157–159	MeOH	$C_{18}H_{15}ClN_4O$
38	4-OAc	C (70)	233–236	EtOH–$CHCl_3$	$C_{17}H_{11}ClN_4O_2$
39	4-OH	*b* (80)	265–268	$MeOCH_2CH_2OH$	$C_{15}H_9ClN_4O$
40	4-$CONH_2$	A (89)	308–209	$HCONMe_2$	$C_{16}H_{10}ClN_5O$
41	3-Me, 4-OMe	A (94)	236–238	EtOH–$CHCl_3$	$C_{17}H_{13}ClN_4O$

[a] All compounds analyzed correctly (±0.3) for C, H, N. [b] By alkaline hydrolysis of compound **38**.

zones **1–14** (Table I), which undergo oxidative cyclization with bromine (method A) or lead tetraacetate (method B) to give 3-aryl-6-chloro-1,2,4-triazolo[3,4-*a*]phthalazine **15–41** (Table II). Lead tetraacetate is preferred for the cyclization of the hydrazones of arylaldehydes substituted with electron-releasing groups. Alternatively, **15–41** can be prepared by acylation and cyclization of 4-chloro-1-phthalazinylhydrazine with equimolar amounts of aroyl-chlorides in boiling dioxane (method C). Reaction of the 6-chloro derivatives **15–41** with a variety of amines provides the corresponding 6-(alkylamino)-3-aryl-1,2,4-triazolo[3,4-*a*]phthalazines **42–87** (Table III). There are two similar methods to carry out this reaction, one of which (D) uses lower reaction temperatures (100 °C), a solvent, and shorter reaction times, and the other (E) uses higher reaction temperatures (140 °C) and longer reaction times without any solvent.

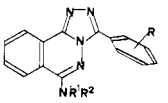
Biological Results and Discussion

Compounds **42–87** were routinely examined in vivo for their anticonvulsant effects in the pentylenetetrazole (PTZ) induced convulsions test (Table IV) and in vitro in the ^{3}H-DZ binding assay (Table IV). The K_i (nM) values were used as a preliminary selection criterion and to direct further synthesis. Thus compounds with K_i values less than or equal to 150 nM were further examined in vitro for differences in their K_i values in the presence and in the absence of GABA (GABA ratio) to differentiate BZ agonist, partial agonist, and antagonist compounds[19] at BZ receptor sites (Table IV). The compounds with a GABA ratio equal to or higher than 1.4 were examined in vivo for their effects in the anticonflict test (Table IV). The compounds that were either inactive in the PTZ test or that had K_i values higher than 150 nM were arbitrarily discarded from further study; compounds **45** and **78** were eliminated because of their insolubility in water or organic solvents, and compound **84** was eliminated because of its K_i value.

Two positions were considered for chemical manipulation, i.e., the 6-position and the 3-phenyl ring of the triazolophthalazine system. Compounds **42**, **57–70** were synthesized to determine how primary, secondary, or tertiary amino groups affect the K_i values. The results of Table IV show that there are two groups of roughly equivalent compounds: those with K_i values below 100 nM, i.e., **42**, **58**, **59**, **64–70**, and those with K_i values above 200 nM, i.e., **57**, **60–63**; primary and secondary amines fall in this latter group, with the exception of compounds **58** and **59**. The 1-pyrrolidino and the *N*,*N*-bis(2-methoxyethyl) groups were chosen as the most suitable substituents at the 6-position, the former because of the low K_i value of compound **42** (15.6 nM), which is equivalent to that of compounds **64**, **66**, **68**, and **69**, and because of the convenience of handling pyrrolidine rather than dimethylamine, ethylmethylamine, morpholine, or piperazine during the reaction or during the workup of the reaction mixtures, and the latter because **67** is the compound with the highest GABA ratio (1.6) in the group of the 3-phenyl-6-substituted-triazolo[3,4-*a*]phthalazines with K_i below 100 nM. The effects of the substitution at the 3-phenyl ring on the K_i value were preliminarily studied by synthesizing the set[16] of 16 compounds, **42–56**, **87**, and by directing further synthesis on the basis of the preliminary indications thus obtained. It is apparent from the K_i values of this first group of compounds (Table IV) that multiple substitution at the 3-phenyl ring as in **49–56**, **87**, bulky lipophilic substituents as in **47**, or strong electron donating groups as in **46** are not conducive to in vitro BZ-receptor binding, and in fact, all these compounds have K_i values equal to or higher than 908 nM. Compound **48** has an affinity higher than expected on the basis of its

(19) Ehlert, F. J.; Roeske, W. R.; Yamamura, H. I. *Life Sci.* **1981**, *29*, 235.

Table III. 6-Amino-3-aryl-1,2,4-triazolo[3,4-*a*]phthalazines



no.	R	NR^1R^2	meth (*Y*, %)	mp, °C	solv	formula[a]
42	H	1-pyrrolidinyl	D (82)	208–209	EtOH	$C_{19}H_{17}N_5$
43	2-Br	1-pyrrolidinyl	E (86)	204–206	EtOH–$CHCl_3$	$C_{19}H_{16}BrN_5$
44	4-CN	1-pyrrolidinyl	E (58)	276–278	EtOH–$CHCl_3$	$C_{20}H_{16}N_6$
45	4-NHAc	1-pyrrolidinyl	E (87)	333–335	$CHCl_3$	$C_{21}H_{20}N_6O$
46	4-NMe_2	1-pyrrolidinyl	E (89)	233–235	EtOH	$C_{21}H_{22}N_6$
47	4-C_6H_5	1-pyrrolidinyl	E (80)	253–254	EtOH–$CHCl_3$	$C_{25}H_{21}N_5$
48	2-Cl, 4-NMe_2	1-pyrrolidinyl	E (80)	216–219	EtOH	$C_{21}H_{21}ClN_6$
49	3,4-Me	1-pyrrolidinyl	E (90)	184–186	EtOH	$C_{21}H_{21}N_5$
50	3-OMe, 4-OC_5H_{11}-*n*	1-pyrrolidinyl	E (94)	176–178	EtOH	$C_{25}H_{29}N_5O_2$
51	3,4-OMe	1-pyrrolidinyl	E (87)	229–231	EtOH	$C_{21}H_{21}N_5O_2$
52	3-NO_2, 4-Cl	1-pyrrolidinyl	D (56)	278–280	EtOH–$CHCl_3$	$C_{19}H_{15}ClN_6O_2$
53	2,4,5-OMe	1-pyrrolidinyl	E (79)	217–218	EtOH	$C_{22}H_{23}N_5O_3$
54	3,5-Br, 2-OEt	1-pyrrolidinyl	E (88)	194–196	EtOH–$CHCl_3$	$C_{21}H_{19}Br_2N_5O$
55	2,4,5-OEt	1-pyrrolidinyl	E (77)	217–218	EtOH	$C_{25}H_{29}N_5O_3$
56	3,4-OMe, 5-Br	1-pyrrolidinyl	E (57)	229–231	EtOH–$CHCl_3$	$C_{21}H_{20}BrN_5O_2$
57	H	NH_2	D (54)	314–316	$MeOCH_2CH_2OH$	$C_{15}H_{11}N_5$
58	H	NHMe	D (76)	318–319	$MeO(CH_2)_2OH$	$C_{16}H_{13}N_5$
59	H	NHEt	D (74)	289–290	$MeOCH_2CH_2OH$	$C_{17}H_{15}N_5$
60	H	NH-*i*-C_3H_7	D (54)	269–270	$MeOCH_2CH_2OH$	$C_{18}H_{17}N_5$
61	H	NH-*n*-C_4H_9	D (75)	254–255	$MeOCH_2CH_2OH$	$C_{19}H_{19}N_5$
62	H	NH-*t*-C_4H_9	E (72)	241–244	EtOH	$C_{19}H_{19}N_5$
63	H	$NHCH_2C_6H_5$	D (88)	283–284	EtOH	$C_{22}H_{17}N_5$
64	H	NMe_2	D (85)	177–178	EtOH	$C_{17}H_{15}N_5$
65	H	NEt_2	D (63)	115–116	EtOH	$C_{19}H_{19}N_5$
66	H	NMeEt	D (56)	114–116	MeOH	$C_{18}H_{17}N_5$
67	H	$N(CH_2CH_2OMe)_2$	E (71)	102–103	AcOEt	$C_{21}H_{23}N_5O_2$
68	H	1-morpholinyl	D (70)	197–198	EtOH	$C_{19}H_{17}N_5O$
69	H	1-piperidinyl	D (67)	198–200	EtOH	$C_{20}H_{19}N_5$
70	H	1-(4-Me-piperazinyl)	D (80)	171–172	EtOH	$C_{20}H_{20}N_6$
71	3-Cl	$N(CH_2CH_2OCH_3)_2$	E (78)	126–227	AcOEt	$C_{21}H_{22}ClN_5O_2$
72	3-Me	$N(CH_2CH_2OMe)_2$	E (78)	104–105	AcOEt	$C_{22}H_{25}N_5O_2$
73	3-CF_3	1-pyrrolidinyl	D (87)	217–219	EtOH–$CHCl_3$	$C_{20}H_{16}F_3N_5$
74	4-Cl	$N(CH_2CH_2OMe)_2$	E (77)	133–135	AcOEt	$C_{21}H_{22}ClN_5O_2$
75	4-F	$N(CH_2CH_2OMe)_2$	E (81)	127–129	AcOEt	$C_{21}H_{22}FN_5O_2$
76	4-Me	$N(CH_2CH_2OMe)_2$	E (80)	122–124	AcOEt	$C_{22}H_{25}N_5O_5$
77	4-OH	$N(CH_2CH_2OMe)_2$	E (70)	179–181	MeOH	$C_{21}H_{23}N_5O_3$
78	4-OH	1-pyrrolidinyl	D (91)	345–348	$HCONMe_2$	$C_{19}H_{17}N_5O$
79	4-OMe	$NHCH_2CH_2OMe$	E (86)	262–264	EtOH–$CHCl_3$	$C_{19}H_{19}N_5O_2$
80	4-OMe	$N(CH_2CH_2OMe)_2$	E (75)	100–101	AcOEt	$C_{22}H_{25}N_5O_3$
81	4-OMe	N(Me)CH_2CH(OH)Me	D (57)	168–169	MeOH	$C_{20}H_{21}N_5O_2$
82	4-OMe	1-pyrrolidinyl	D (93)	228–229	$MeOCH_2CH_2OH$	$C_{20}H_{19}N_5O$
83	4-O-*i*-Pr	$N(CH_2CH_2OMe)_2$	D (79)	90–92	Et_2O	$C_{24}H_{29}N_5O_3$
84	4-NMe_2	$N(CH_2CH_2OMe)_2$	E (74)	114–115	AcOEt	$C_{23}H_{28}N_6O_2$
85	4-$CONH_2$	1-pyrrolidinyl	E (60)	305–307	EtOH	$C_{20}H_{18}N_6O$
86	4-COOH	1-pyrrolidinyl	*b* (60)	340	EtOH	$C_{20}H_{17}N_5O_2$
87	3-Me, 4-OMe	$N(CH_2CH_2OMe)_2$	E (71)	115–17	AcOEt	$C_{23}H_{27}N_5O_3$

[a] All compounds analyzed correctly (±0.3) for C, H, N. [b] By alkaline hydrolysis (KOH–*tert*-butyl alcohol) of compound **44**.

disubstitution on the 3-phenyl ring. It must be noted however that **48** cannot be easily compared with the other polysubstituted compounds as it is the only one of the series with a 2,4-type disubstitution on the 3-phenyl ring, and this might imply that an unsubstituted meta position is essential for the binding.

The K_i values of this set of compounds were analyzed by the manual simplex method of Darvas[17] and a region between $\pi = +0.8$ and $\pi = -0.6$ and $\sigma = +0.8$ and $\sigma = -0.2$ was identified as one likely to contain a maximum of affinity; on the basis of this procedure the second set of compounds, **71–86**, was synthesized to refine the substitution on the aromatic ring and on the 6-position. It is apparent from the data of Table IV that substitution on the 3-phenyl ring of a 4-OCH_3, 4-OH, 4-CH_3, 4-Cl, or 4-F group (compounds **74–82**) is conducive to high affinity. The 4-OCH_3 and the 4-OH groups are particularly favorable substituents, see for example **78** and **82** vs **42** and **77** and **80** vs **67**.

Compounds **42**, **64**, **66**, **68**, **69**, **74**, **75**, **77–82** all have K_i values equal to or less than 20.8 nM. A second group of compounds with K_i values between 36 and 102 nM, i.e., compounds **44**, **45**, **58**, **59**, **65**, **67**, **70**, **76**, with an unsubstituted 3-phenyl ring, or with a 4-CH_3, 4-NHAc, or 4-CN substitution on the 3-phenyl ring can be evidentiated (Table IV). With regard to substitution on the 6-position and on the basis of the K_i values of compounds **42–87**, it is apparent that a tertiary amino group is preferable to a secondary amino group as **79** is the only secondary amine that ranks in the group of compounds with K_i values up to 20.8 nM (see also **64** vs **58** and **80** vs **79**) and that the *N*,*N*-bis(2-methoxyethyl)amino substituent gives compounds with a slightly better affinity for the BZ receptors than the pyrrolidino group (**80** vs **82** and **77** vs **78**). The *N*,*N*-bis(2-methoxyethyl)amino group is the only one among those tried that gives compounds with a GABA ratio equal to or higher than 1.5, i.e., **67**, **75**, **76**, **80**, and **83**. The data at hand do not allow a more detailed structure–affinity analysis.

Many of the compounds of Table IV showed affinities for the BZ cerebral receptors labeled with ^{3}H-DZ comparable to those of the reference drugs, i.e., ethyl 8-fluoro-

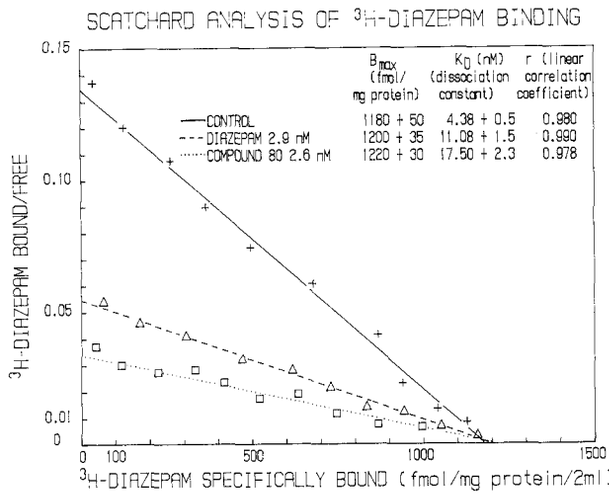


Figure 1. Regression lines as the mean of three experiments each done in triplicate. See the Experimental Section. Key: B_{max} = maximum number of specific binding sites; K_D = dissociation constant; r = correlation coefficient.

5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-*a*]1,4-benzodiazepine-3-carboxylate (Ro 15-1788)[18] (Chart I), Cl-218872, diazepam, and chlordiazepoxide. On the basis of their K_i values, compounds **42**, **44**, **45**, **58**, **59**, **64–70**, **74–83**, **85** were selected to be examined in vitro for differences in their K_i values in the presence and in the absence of GABA (GABA ratio). The GABA ratio of these compounds ranged from 0.9 to 1.8, indicating the presence of BZ agonist, partial agonist, and antagonist compounds;[19] however, the majority of these values was comprised between 1.1 and 1.3 (antagonist and partial agonist compounds) similarly to what we had previously observed for the 3-aryl-6-(alkylamino)pyridazino[4,3-*c*]isoquinolines.[7] Compounds **42**, **44**, **45**, **58**, **59**, **64–66**, **68–70**, **77–79**, **82**, and **85** with GABA ratios close to 1 resembled the antagonist Ro 15-1788 and were inactive in counteracting the PTZ-induced convulsions at the screening dose (1/4–1/6 LD_{50}) (Table IV). Compounds **42**, **64**, and **82**, with GABA ratios of 1.1, were examined in the traction test (mice, ip) for their antagonism to the muscle relaxant action of diazepam (Table V). Compounds **42**, **64** (10 mg/kg, ip), and **82** (60 mg/kg, ip) antagonized the muscle relaxant effects induced by pretreatment with diazepam (3 mg/kg, ip) to the same extent as 0.2 mg/kg, po, of the antagonist Ro 15-1788, used as a reference compound.

Compounds **67**, **76**, **80**, **81**, and **83** had a moderate anticonvulsant activity with ED_{50} values (mice, ip) (Table IV) less than 35 mg/kg, ip, in the PTZ anticonvulsant test and in agreement with their GABA ratios (1.4–1.9). These compounds were evaluated for their effects on operating behavior in the Vogel conflict scheme (rat, ip) (Table IV), and compound **80** was selected for further evaluation on the basis of the results of this test.

Compound **80** had a K_i (nM) in vitro comparable to that of diazepam (Table IV) and about $^1/_{50}$ that of chlordiazepoxide and more than $^1/_{100}$ that of CL 218,872 with a GABA ratio typical of an agonist (Table IV). Scatchard analysis[20] (Figure 1, Table VIII) was applied to saturation studies of the specific ^{3}H-DZ binding to synaptosomal membrane preparations in the presence and in the absence of 2.6 nM compound **80** and 2.9 nM nonradioactive diazepam to determine whether compound **80** and ^{3}H-DZ share the same receptor sites (competitive antagonism) or not (noncompetitive antagonism). In the presence of both drugs, there was a significant reduction ($p \leq 0.01$) in the affinity of the complex (^{3}H-DZ/BZ-receptor), since its apparent dissociation constant (K_d) was increased to 11.8 nM (diazepam) and 17.50 nM (**80**) from the control value (4.38 nM) while the maximum number of available binding sites (B_{max}) was unaffected by either. The different curves (Figure 1) were compared by testing the significance of the differences in slopes and intercepts, according to Colton.[21] This analysis showed that the intercepts on the ordinates (affinities) were significantly different ($p \leq 0.01$), whereas the intercepts on the abscissa (B_{max}) were not. The three regression curves were neither parallel nor coincident ($p \leq 0.01$), indicating that compound **80** and diazepam act through the same mechanism in the inhibition of ^{3}H-DZ specific binding and that they share the same receptor sites (competitive antagonism).

The effects of compound **80** in anticonvulsion (strychnine-induced lethality, maximal electroshock induced convulsions, bicuculline induced convulsions) and motor coordination (rotarod) tests in mice and rats (Table VI), on "conditioned behavior" (CR_2) (Table VI) and "normal behavior" (Table VII) of rats, on "normal behavior" of cats and dogs, and on "aggressive monkeys" (vide infra) were evaluated. Compound **80** was active in the Vogel conflict test (rat) with a minimal effective dose (MED) of 18.7 (7.5–30) mg/kg, po, vs 1.9 (0.75–3.0) mg/kg, po, for diazepam and in the conditioned avoidance response test (CR_2) at 10 mg/kg, ip, vs 10 mg/kg, ip, for diazepam and 7.65 mg/kg, ip, for chlordiazepoxide (Table VI). These values are of particular interest when compared to the ED_{50} in the rotarod test (Table VI). In the rotarod test compound **80** had an ED_{50} of 300 mg/kg, po, in the rat, and of 60 mg/kg, ip, in the mouse (Table VI), this latter value being between 60 times greater than that for diazepam (1 mg/kg, ip) and 6 times those for chlordiazepoxide (11 mg/kg, ip) and CL 218872 (10 mg/kg, ip). It is important to note that the ratio of the MED values for the rotarod and Vogel tests (rat) for compound **80** is about 100, whereas this ratio is about 20 for diazepam, indicating a lesser incidence of aspecific CNS side effects for compound **80** than for the reference drug.

Toxicity and depressive effects on the normal behavior of rodents (mouse and rat) were more evident in the mouse than in the rat (Table VII). Dose-related side effects lasting up to 5 h (sedation, motor uncoordination, ptosis, muscular hypotonia) were observed in cats (two animals/dose) at all doses (30, 10, 6, 1 mg/kg, po) and to a lesser extent (slight sedation and motor uncoordination) in dogs (two animals/dose) at 10 and 50 mg/kg, po.

Compound **80**, unlike diazepam and chlordiazepoxide, had poor activity in protection against the strychnine- and MES-induced convulsions (Table VI), whereas it had an activity comparable to that of chlordiazepoxide in protection against the bicuculline-induced convulsion test. Finally administration of **80** to aggressive cynomolgus monkeys (three animals/dose; 20 and 10 mg/kg, po) did not have any taming effect, whereas diazepam had taming effects from 0.25 to 2 mg/kg, po with signs of motor incoordination from 1 mg/kg, po.

The selectivity of the anticonvulsant effects of **80** in mice and the lack of taming effects in aggressive monkeys are an indication of a higher degree of selectivity of action than that of diazepam. The profile of compound **80** and in general of the 3-aryl-6-(alkylamino)-1,2,4-triazolo[3,4-*a*]-phthalazines resembles that of the analogous 3-aryl-6-

(20) Scatchard, G. *Ann. N. Y. Acad. Sci.* **1949**, *51*, 660.

(21) Colton, T. *Statistics in Medicine*; Little, Brown and Co.: Boston, 1974; pp 189–218.

(alkylamino)pyridazino[4,3-c]isoquinolines, especially with regard to the presence of both BZ agonistic and antagonistic compounds in the same class.

Conclusions

Chemical modification of the triazolophthalazine system provides BZ receptor ligands that can be classified, on the basis of in vitro and in vivo results, from agonists to antagonists. Compound **80** shows important differences from DZ. Dissociation of the "anxiolytic" effects and of the sedative side effects is suggested in the rat by the difference between the dose that affects conflict and conditioned behavior and the dose that affects motor coordination. Whether this dissociation is a pharmacological characteristic of the compound of possible clinical importance or a species-dependent phenomenon requires further study, since sedation and motor incoordination of different degrees were observed in mice, cats, and dogs, although in tests not specifically designed to differentiate between doses causing antianxiety and sedative and muscle relaxant effects.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes with a Büchi or, for melting points above 250 °C, with a Gallenkamp apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 137 spectrometer in Nujol mull. Mass spectra were taken on a Hitachi RMU 6L instrument and nuclear magnetic resonance spectra were recorded on a Bruker WH-270 spectrometer. The IR, ^{1}H NMR, and mass spectra were in agreement with the proposed structures. Correct elemental analyses (C, H, N) (±0.3% from the theoretical value) were obtained for all compounds. Elemental analyses were carried out at the Analytical Laboratories of the Lepetit Research Center. Starting materials are known compounds, and most of them are commercially available. 4-Methoxy-3-(pentyloxy)benzoyl chloride is new, and its synthesis is reported.

Arylaldehydes (4-Chloro-1-phthalazinyl)hydrazones 1–14 (Table I). General Procedure. (4-Chloro-1-phthalazinyl)-hydrazine[15] (0.1 mol) was dissolved in 0.1 N HCl (1000 mL). The desired aromatic aldehyde (0.1 mol) was added drop by drop, and the mixture heated at 60 °C, with stirring, for 10 min and then cooled to room temperature. The mixture was brought to a pH value of 7.5 by addition of diluted aqueous NaOH, stirred a few minutes, and filtered. The collected solid was washed with water and recrystallized from the solvents listed in Table I. The mass spectra of **1–14** show an abundant molecular ion with a diagnostic fragment at m/e 205.

3-Aryl-6-chloro-1,2,4-triazolo[3,4-*a*]phthalazines 15–41 (Table II). Method A. Bromine (0.105 mol) in acetic acid (60 mL) was added drop by drop to a mixture of the desired arylaldehyde (4-chloro-1-phthalazinyl)hydrazone (0.1 mol) and sodium acetate (0.3 mol) in acetic acid (900 mL). The resulting mixture was allowed to react at room temperature, with stirring, for 1 h and was then poured into cold water (5000 mL). The precipitate was collected by filtration, washed with 10% aqueous $NaHSO_3$ (2 × 200 mL) and then with water, and finally recrystallized from the solvents of Table II to give **15, 18, 20, 26–29, 31, 35, 40, 41.**

Method B. Lead tetraacetate (0.12 mol) was added to a stirred suspension of the desired arylaldehyde (4-chloro-1-phthalazinyl)hydrazone (0.1 mol) in acetic acid (400 mL), the temperature was raised to 45 °C, and the hydrazone was dissolved. The reaction mixture was cooled and maintained for 1 h at room temperature, poured into cold water (2000 mL), and neutralized with solid sodium bicarbonate. The precipitate was collected by filtration, washed with water, and recrystallized to yield **19, 21.**

Method C. A mixture of (4-chloro-1-phthalazinyl)hydrazine (0.1 mol) and triethylamine (0.12 mol) in dry dioxane (250 mL) was treated with the desired acyl chloride (0.12 mol) in dry dioxane (50 mL). The mixture was refluxed with stirring for 5 h. After cooling, the solvent was evaporated under vacuum, and the residue was triturated with water. The insoluble material was collected, washed with water, and recrystallized to yield **16, 17, 22–25, 30, 32–34, 36–38.**

Table IV. K_i (nM) Values in the ^{3}H-DZ in Vitro Binding Assay of Compounds **42–87**: GABA Ratio and Anti-PTZ and Anticonflict Effects of Some Selected Compounds

no.	LD_{50}, mg/kg, ip (mice)	K_i,[a] nM	GABA ratio[b]	anti-PTZ[c] ED_{50}, mg/kg, ip (mice)	anticonflict[d] MED, mg/kg, ip (rat)
42	400	15.6	1.1	≥75	NT
43	600	760		≥100	NT
44	600	102	1.1	≥100	NT
45	600	36	1.1	≥100	NT
46	300	1720		≥75	NT
47	400	≥3000		≥75	NT
48	600	393		≥100	NT
49	300	1970		≥50	NT
50	200	≥3000		≥50	NT
51	500	≥3000		≥100	NT
52	200	908		≥50	NT
53	300	1930		≥50	NT
54	600	≥3000		≥100	NT
55	300	≥3000		≥50	NT
56	600	≥3000		≥100	NT
57	600	243		≥100	NT
58	300	46.3	1.0	≥60	NT
59	200	37.6	0.9	≥50	NT
60	400	306		≥75	NT
61	600	243		≥100	NT
62	300	565		≥50	NT
63	400	243		≥75	NT
64	400	20.8	1.1	≥75	NT
65	600	41	0.9	≥100	NT
66	300	14.4	1.1	≥50	NT
67	200	66	1.6	34.2 (16.5–44.8)	15
68	300	14	0.95	≥100	NT
69	600	16	1.0	≥100	NT
70	300	37.7	0.9	≥50	NT
71	600	307		≥100	NT
72	300	181		≥100	NT
73	600	≥3000		≥100	NT
74	600	17	1.3	60.1 (51.3–69.1)	NT
75	600	20	1.5	60.1 (51.3–69.1)	NT
76	400	44	1.9	20.4 (13.1–27.7)	10
77	600	4.1	1.0	≥50	NT
78	600	11	1.1	≥100	NT
79	200	5.4	1.1	≥50	NT
80	300	2.6	1.83 ± 0.06	6.2 (4.5–8.7)	3.5
81	300	17	1.4	9.8 (6.7–12.9)	10
82	600	3.9	1.1	≥100	NT
83	600	142	1.8	20.7 (14.1–27.3)	10
84	200	2650		20 (12.9–27.1)	NT
85	200	113	1.1	≥50	NT
86	400	≥3000		≥75	NT
87	400	1820		≥100	NT
DZ		2.9	1.98 ± 0.03	0.2 (0.11–0.28)	0.25
		1.8	0.90 ● 0.04	inactive	inactive
		144	1.93 ± 0.05	2.2 (1.69–3.08)	3
		323	1.17 ± 0.03	3	3

[a] $K_i = IC_{50}/(1 + C/K_d)$ in which C = concentration of ^{3}H-DZ (1.2 nM) and K_d = apparent dissociation constant of the complex ^{3}H-DZ/BZ receptors (3.5 nM). IC_{50} values were assessed from at least six concentrations in triplicate, and the determinations were repeated at least twice. [b] IC_{50} (compound)/IC_{50} (compound + GABA 10^{-4} M). [c] Dose that prevented tonic extensor seizures and death in 50% of the mice; 10 animals/dose used; 95% confidence limits in parentheses. [d] Minimal effective dose that significantly (Mann–Whitney U test) increased the number of shocks in comparison with controls; 10 animals/dose used. [e] Ro 15-1788 was kindly donated by Dr. W. Haefely, Hoffman-La Roche, Basle, Switzerland. [f] Cl-218872 was synthesized in the Lepetit Res. Lab by the method in the literature.[22] Ro 15-1788[e] chlordiazepoxide Cl-21872[f]

3-Aryl-1,2,4-triazolo[3,4-*a*]phthalazin-6-amines 42–87 (Table III). Method D. A mixture of the desired 3-aryl-6-

Table V. Antagonism of Diazepam Muscle Relaxant Effect by Compounds **42, 64,** and **82** (Traction Test, Mice)

compd	dose,[a] mg/kg, ip	fallen/treated
42	10	4/10
	30	2/10
64	10	5/10
	30	2/10
82	60	5/10
Ro 15-1788	0.2[b]	5/10
	0.4[b]	2/10
diazepam	3	8/10

[a] Compounds were administered after pretreatment (15 min) with 3 mg/kg, ip, of diazepam. [b] Oral administration.

chloro-1,2,4-triazolo[3,4-*a*]phthalazine (0.03 mol), the desired amine (0.07 mol), catalytic amounts of potassium iodide, and one drop of concentrated hydrochloric acid in ethanol (40 mL) was heated at 100 °C in a steel bomb for 8 h. After cooling, the solvent was removed, and the residue was triturated with water. The insoluble material was separated by filtration, washed with water, and recrystallized to yield **42, 52, 57–61, 63–66, 68–70, 73, 78, 81–83.**

Method E. The desired 3-aryl-6-chloro-1,2,4-triazolo[3,4-*a*]-phthalazine (0.03 mol) and the desired amine (30 mL) were heated for 30 h at 140 °C in a steel bomb. After cooling, the excess reagent was distilled off under vacuum, and the solid was triturated with water and recrystallized to yield **43–51, 53–56, 62, 67, 71, 72, 74–77, 79, 80, 84, 85, 87.**

3-Methoxy-4-(pentyloxy)benzoyl Chloride. Methyl 4-hydroxy-3-methoxybenzoate[25] (24.5 g, 0.135 mol), powdered potassium carbonate (19 g, 0.137 mol), and pentyl bromide (22.6 g, 0.150 mol) in acetone (100 mL) were boiled for 16 h with vigorous stirring. The residue obtained after filtration of the salts and evaporation of the acetone was dissolved in diethyl ether and washed twice with 1% aqueous sodium hydroxide. The residue obtained after evaporation of the ether was recrystallized from petroleum ether to yield methyl 3-methoxy-4-(pentyloxy)benzoate (29.5 g, 87%): mp 37–38 °C; IR 1710 (C=O), 1250, 1030 (C—O—C), 780, 760, 730 (CH arom) cm^{-1}. Alkaline hydrolysis of this material gave the corresponding acid, mp 134–136 °C (EtOH) (20.8 g), which was treated with thionyl chloride (15 mL) in dry benzene at 80 °C for 4 h. After the usual workup, the oily residue was distilled to yield the title compound (21 g, 94%): bp (0.1 mmHg) 128–130 °C; IR 1760 (COCl) cm^{-1}.

Biological Test Procedures. Diazepam (Dz) was purchased from FIS, Ro 15-1788 was kindly donated by Dr. W. Haefely (Hoffmann-La Roche, Basle), and Cl 218872 was synthesized in our laboratories by the published procedure.[22] ^{3}H-DZ (specific activity 76.8 Ci/mmol) and [^{3}H]Flunitrazepam (specific activity 70.2 Ci/mmol) were purchased from New England Nuclear, Boston, MA. The radioactivity was measured in a Packard 460 C liquid scintillation spectrometer. The homogenates were obtained with a Brinkman-Polytron PT10 microhomogenizer, setting 7 for 20 s.

Benzodiazepine-Receptor Binding in Vitro. [^{3}H]Diazepam binding studies were carried out according to the method of Mohler and Okada,[23] incubating ^{3}H-DZ (0.65–1.20 nM) with membranes isolated from synaptosomes of rat forebrain. Specific binding was determined by subtracting the binding in the presence of 3 μM nonradioactive DZ from the binding in the presence of ^{3}H-DZ alone and represented 95% of total binding after [(total binding) − (nonspecific binding)]. The concentrations of the test compounds that caused 50% inhibition of the specific ^{3}H-DZ binding (IC_{50}) were determined at least in triplicate. The inhibition curves were transformed into straight lines by the probit analysis.[24] In saturation studies, 10 different ^{3}H-DZ concentrations from 0.05 to 40 nM were incubated in triplicate in the absence and presence of the compounds at their respective K_i concentrations. The nonspecific binding was determined in the presence of 3 μM nonradioactive DZ in triplicate for each concentration of ^{3}H-DZ. The statistical significance of differences ($p \leq 0.01$) in slopes and intercepts were determined by the method of Colton.[21] The GABA ratio for the rat forebrain was determined by the method of Wastek et al.[25] One milliliter of membrane suspension was incubated in triplicate with 0.4 nM [^{3}H]flunitrazepam and various concentrations of the ligands, with and without 0.1 mM GABA, for 20 min at 37 °C. The binding in the presence of 1 μM nonradioactive Clonazepam (nonspecific binding) was subtracted from the binding in the absence of excess Clonazepam to obtain the specific binding. IC_{50} values were the concentrations of test compound that caused 50% inhibition of specific [^{3}H]flunitrazepam binding. The Student's t test was used to evaluate the statistical significance of differences between IC_{50} values. The in vitro binding data were calculated on an Apple II microcomputer with the Receptor Program described by Benfenati and Guardabasso.[26]

Acute Toxicity. Test compounds were dispersed in 0.5% methocel at a volume of 10 mL/kg and administered ip to male CD1 mice, in groups of three for each dose, i.e., 600–300–100 mg/kg. The animals were observed for 1–5 days, and the LD_{50} values were graphed.

Anti-Pentylenetetrazole-Induced Convulsion Test. The method described by Berger[27] was employed. Test compounds dispersed in 0.5% methocel at a volume of 10 mL/kg were given ip to 10 male CF1 mice (19–21 g) maintained in a sound proof room at 21–22 °C. Pentylenetetrazole (140 mg/kg) was administered sc as an aqueous solution 30 min later. The control-group animals treated with the vehicle and pentylenetetrazole developed convulsions and died within 30 min. The number of survivors at 2 h in the experimental groups was recorded. ED_{50}, the dose that prevented tonic extensor seizures in 50% of the mice, was calculated by the probit analysis of Finney,[28] with the System 30/IBM program, Scientific Subroutine Package.

Vogel Conflict Procedure. The Vogel procedure for unconditioned rats, as modified by Lippa et al.,[29] was used. Naive male Wistar rats deprived of food (24 h) and water (48 h) were placed in a black Plexiglas 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. The 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 ($p \leq 0.05$) increased the number of shocks in comparison with the controls, was determined. The significance was assessed by the Mann–Whitney U-test.[30]

Interaction with Maximal Electroshock (MES). The effects of the test and reference compounds on electroshock were evaluated as the antagonism to the tonic phase of MES-induced convulsions. The animals (male CF-1 mice) were considered protected when no extension of the hind legs was observed. The apparatus to produce electroshock was constructed in the Lepetit Laboratories. MES was produced by the procedure of Swinyard.[31] The stimulus was applied 30 and 90 min after treatment with the test or reference compounds by means of corneal platinum electrodes. A sinusoidal current of 50 Hz, 200 ms and 35 effective mA, was used. ED_{50} values were calculated by the Finney probit analysis.[28]

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Table VI. Conditioned Avoidance Response Activity (CR_2), Anticonvulsant Activity, and Activity in the Rotarod Test of Compound **80**

compd	CR_2, mg/kg, ip (rat) block/treat[a]	ED_{50}, mg/kg, ip (mouse) strych leth	MES	bicuculline	rotarod: ED_{50}, mg/kg (mouse, ip)	(rat, po)
80	10 (4/10) 20 (10/10)	50	50	4	60	300
diazepam	10 (5/10)	3	14.2	1.4	1	15
chlordiazepoxide	*b*	7.5	23	4.7	11	68.4
Cl-218872	*c*	30	30		10	

[a] Number of rats with block of conditioned response/number of treated rats in parentheses. [b] ED_{50} = 7.65 (6.32–9.25) mg/kg, ip. [c] Not tested.

Table VII. Effects of **80** on Normal Behavior and on Mortality of Mice and Rats

test	mouse (mg/kg, ip)[a] MED	ED	rat (mg/kg, ip)[a] MED	ED
motility[b]	1–10	30–60	60–300	1000
motor coordination[c]	1–6	30–60	60–100	600
body tone[d]	1–6	30–100	60–300	600
mortality	300[e]	600[f]	1000[e]	

[a] MED = minimal effective dose; ED dose effective in 100% of test animals. [b] Decrease in spontaneous motility. [c] Impairment of righting reflex and ataxia. [d] Decrease in muscle tone. [e] 1/3 animals died. [f] 3/3 animals died.

Table VIII. Scatchard Analysis of [^{3}H]Diazepam Binding of Compound **80** and Diazepam

compd	Y[a]	X[b]	B_{max}, fmol/mg of protein	K_D, nM	r
80 (2.6 nM)	0.0370	41.66	1220 ± 30	17.50 ± 2.3	0.978
	0.0300	118.33			
	0.0272	223.33			
	0.0283	333.33			
	0.0233	416.66			
	0.0171	520.00			
	0.0191	633.33			
	0.0116	745.83			
	0.0076	866.66			
	0.0066	993.33			
diazepam	0.0538	65.00	1200 ± 35	11.08 ± 1.5	0.990
(2.9 nM)	0.0458	171.66			
	0.0408	303.33			
	0.0321	470.00			
	0.0280	616.66			
	0.0213	728.33			
	0.0140	833.33			
	0.0125	941.66			
	0.0067	1048.33			
	0.0033	1155.00			
control	0.1370	38.33	1180 ± 50	4.38 ± 0.5	0.980
	0.1200	125.00			
	0.1075	261.66			
	0.0895	365.00			
	0.0743	495.83			
	0.0608	677.50			
	0.0411	866.66			
	0.0230	938.33			
	0.0133	1040.00			
	0.0083	1125.00			

[a] Y = [^{3}H]diazepam bound/free. [b] X = [^{3}H]diazepam specifically bound (fmol/mg of protein per 2 mL).

Anti-Bicuculline-Induced Convulsion Test. The method described by DelaMora was employed.[32] An aqueous solution of bicuculline was administered subcutaneously to male CD1 mice, at the dose of 2 mg/kg, 30 min after treatment with test compounds and via the experimental procedure described for the pentylenetetrazole test.

Anti-Strychnine-Induced Convulsion Test. The technique of Mustala and Penttilä[33] was employed, with several modifications. The protective effects were evaluated against effectively lethal doses of strychnine sulfate. Male CF-1 mice, 10 animals/dose, housed in individual cages, were treated with the test or reference compounds 30 min before subcutaneous injection of 1.5 mg/kg strychnine sulfate. Control animals were pretreated with saline only and developed convulsions followed by death within 30 min after the injection of strychnine sulfate. Results are expressed as the number of the surviving animals 90 min after the injection of the convulsant over the number of the treated animals.

Rotarod Test. The effect on motor coordination in male Wistar rats was determined by the method of Dunham and Miya.[34] 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/dose were placed on the rod 30 and 60 min after treatment with test compounds suspended in 0.5% methocel at a volume of 4 mL/kg. The number of animals that fell off the rod during the 5-min session was recorded.

Traction Test. Muscle relaxation was evaluated by the method of Julou-Courvoisier as described by Boissier et al.[35] The apparatus consisted of a metal rod 2.5 mm in diameter and 30 cm in length fixed horizontally 15 cm above the platform. Male CD1 mice were let hang freely from the rod by their forepaws. Normal animals climb on the rod within 4 s, to hang by all four paws, whereas animals with impaired muscle 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 80% of the animals 30 min after treatment. Test compounds, suspended in 0.5% methocel at a volume of 10 mL/kg were administered intraperitoneally at two dose levels, to mice pretreated with 3 mg/kg DZ, 10 animals/dose, 15 min after the DZ treatment. Fifteen minutes later, the mice were suspended from the rod by their forepaws, and the number falling from the rod was recorded.

Free Behavior of Various Animal Species. Behavioral changes induced by the test compounds were studied in male CF-1 mice, male Wistar rats, and mongrel dogs. In mice and rats the directly observable alterations in certain behavioral, neurophysiological, and neurovegetative parameters were studied as described by Irwin.[36] Three animals/dose group, treated with different doses of the test compound, were observed continuously for 8 h after treatment and twice a day for 5 days afterward. The same observation criteria were employed for the other species, with suitable modifications, focusing on behavior patterns and reactivity to the environment and to other animals of the same species, in the presence and in the absence of the observer. Observations began immediately after treatment and continued until the effect of the treatment disappeared.

Taming Effect. The taming effect is the disappearance of manifestations of hostility against the experimenter without any signs of impairment of behavior. Male macaca cynomolgous monkeys, 4–5.3 kg, were housed and observed in individual cages. The animals demonstrated hostility against the observer before the treatment. All animals that responded to the test drugs were tested again 1 week later with vehicle to rule out false positives. Compounds were given orally by gastric tube, as suspensions in

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1 mL/kg of 0.5% aqueous methocel.

Conditioned Avoidance Response (CR_2). The method of Cook and Weidley[37] was employed, with some modifications of equipment and of interpretation.[38-40] Male Wistar rats were used. The apparatus consisted of a cubic Plexiglas box with metal grid floor connected to a source of electric current and with an insulated wooden pole fitted to the center of the cover and easily accessible to the animals. Acoustic stimulation was provided by a small buzzer fixed in the box. A wooden lining provided sound- and light-proofing. Electrical and acoustic stimulation were under the control of the investigator. The animals were trained to avoid electric shock by jumping onto the pole. The response to electrical stimulus is classed as an unconditioned reflex (UR), the response to the acoustic stimulus is a conditioned response of the first order (CR), and the jump onto the pole during the silent period preceding the acoustic stimulus is a conditioned response of the second order (CR_2). CR_2 is qualitatively different from CR and denotes an emotional state or a state of altered reactivity to environmental stimuli. Changes, if any, in the conditioned behavior pattern (deconditioning), were first recorded 30 min after treatment with the test compound and then every 30 min, until the disappearance or obvious regression of the affects of treatment. Results are expressed as the number of animals that did not show CR_2 response over the number treated at that dose level.

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Registry No. 1, 56813-52-6; 2, 87539-61-5; 3, 87539-65-9; 4, 87539-59-1; 5, 87539-66-0; 6, 87539-60-4; 7, 87539-62-6; 8, 87539-64-8; 9, 87539-63-7; 10, 113628-51-6; 11, 87539-70-6; 12, 87539-69-3; 13, 87539-58-0; 14, 87539-68-2; 15, 56813-54-8; 16, 87539-71-7; 17, 87539-77-3; 18, 113628-52-7; 19, 113628-53-8; 20, 113628-54-9; 21, 113628-55-0; 22, 87539-73-9; 23, 87539-76-2; 24, 87539-78-4; 25, 87539-75-1; 26, 113628-56-1; 27, 113628-57-2; 28, 113628-58-3; 29, 113628-59-4; 30, 87539-81-9; 31, 113628-60-7; 32, 87539-79-5; 33, 87539-72-8; 34, 87539-74-0; 35, 113628-61-8; 36, 98123-77-4; 37, 113628-62-9; 38, 87539-80-8; 39, 113628-63-0; 40, 113628-64-1; 41, 113628-65-2; 42, 87539-84-2; 43, 87540-03-2; 44, 87540-05-4; 45, 87540-06-5; 46, 87540-07-6; 47, 87540-04-3; 48, 87540-10-1; 49, 87540-08-7; 50, 87540-11-2; 51, 87540-09-8; 52, 87540-12-3; 53, 113628-66-3; 54, 87540-17-8; 55, 87540-16-7; 56, 87540-18-9; 57, 87539-89-7; 58, 87539-90-0; 59, 87539-91-1; 60, 87539-92-2; 61, 113628-67-4; 62, 87539-95-5; 63, 87539-93-3; 64, 87539-96-6; 65, 87539-97-7; 66, 87540-01-0; 67, 87539-82-0; 68, 87539-85-3; 69, 87539-98-8; 70, 87539-99-9; 71, 87540-37-2; 72, 87540-38-3; 73, 87540-20-3; 74, 87540-36-1; 75, 113628-68-5; 76, 87540-39-4; 77, 113628-69-6; 78, 87540-29-2; 79, 113628-70-9; 80, 87540-22-5; 81, 87539-86-4; 82, 87540-02-1; 83, 113628-71-0; 84, 113628-72-1; 85, 87540-31-6; 86, 87540-32-7; 87, 113628-73-2; I, 66548-69-4; DZ, 439-14-5; Ro15-1788, 78755-81-4; PhCHO, 100-52-7; *p*-$AcNHC_6H_4CHO$, 122-85-0; *p*-$NMe_2C_6H_4CHO$, 100-10-7; *p*-PhC_6H_4CHO, 3218-36-8; 2-Cl-4-$NMe_2C_6H_3CHO$, 1424-66-4; 2,4,5-$(OMe)_3C_6H_2CHO$, 4460-86-0; 3,5-$(Br)_2$-2-$OEtC_6H_2CHO$, 61657-67-8; 2,4,5-$(OEt)_3C_6H_2CHO$, 67827-54-7; 3,4-$(OMe)_2$-5-BrC_6H_2CHO, 6948-30-7; 3-Me-4-$OMeC_6H_3CHO$, 32723-67-4; *p*-MeC_6H_4CHO, 104-87-0; *m*-MeC_6H_4CHO, 620-23-5; *p*-$OMeC_6H_4CHO$, 123-11-5; *p*-$H_2NCOC_6H_4CHO$, 6051-41-8; *o*-BrC_6H_4COCl, 7154-66-7; *p*-CNC_6H_4COCl, 6068-72-0; 3,4-$(Me)_2C_6H_3COCl$, 21900-23-2; 3,4-$(OMe)_2C_6H_3COCl$, 3535-37-3; 3-NO_2-4-ClC_6H_3COCl, 38818-50-7; *m*-ClC_6H_4COCl, 618-46-2; *m*-$F_3CC_6H_4COCl$, 2251-65-2; *p*-ClC_6H_4COCl, 122-01-0; *p*-FC_6H_4COCl, 403-43-0; *p*-$MeOC_6H_4COCl$, 100-07-2; *p*-*i*-$PrOC_6H_4COCl$, 36823-82-2; *p*-$AcOC_6H_4COCl$, 27914-73-4; NH_3, 7664-41-7; $MeNH_2$, 74-89-5; $EtNH_2$, 75-04-7; *i*-$PrNH_2$, 75-31-0; $BuNH_2$, 109-73-9; *t*-$BuNH_2$, 75-64-9; $PhCH_2NH_2$, 100-46-9; $HNMe_2$, 124-40-3; $HNEt_2$, 109-89-7; HN(Et)Me, 624-78-2; N-$((CH_2)_2OMe)_2$, 111-95-5; $H_2N(CH_2)_2OMe$, 109-85-3; HN(Me)-$CH_2CH(OH)Me$, 16667-45-1; 3-OMe-4-$O(CH_2)_4MeC_6H_3CO_2H$, 95459-64-6; (4-chloro-1-phthalazinyl)hydrazine, 51935-42-3; 3-methoxy-4-(pentyloxy)benzoyl chloride, 113628-74-3; methyl 4-hydroxy-3-methoxybenzoate, 3943-74-6; pentylbromide, 110-53-2; methyl 3-methoxy-4-(pentyloxy)benzoate, 113628-75-4; pyrrolidine, 123-75-1; morpholine, 110-91-8; piperidine, 110-89-4; 1-methylpiperazine, 109-01-3; chlordiazepoxide, 58-25-3.

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