Z-D-Leu-CH=N_2, 118248-00-3; Z-β-homo-D-Leu-OMe, 118248-01-4; BOC-D-Trp-OSu, 22220-11-7; Z-Asp(OBu-t-)-OH, 5545-52-8; Z-Asp(OBu-t)-CH=N_2, 118248-02-5; Z-β-homo-Asp(OBu-t-)-OMe, 83436-44-6; BOC-Leu-OSu, 3392-09-4; H-Leu-β-homo-Asp-Phe-NH_2·TFA, 118248-04-7; Z-D-Asp(OBu-t)-OH, 71449-08-6; Z-D-Asp(OBu-t)-CH=N_2, 118248-05-8; Z-β-homo-D-Asp(OBu-t-)OMe, 118248-06-9; BOC-D-Leu-OSu, 60111-76-4; Z-Phe-OH, 1161-13-3; Z-Phe-CH=N_2, 15196-02-8; Z-β-homo-Phe-OMe, 97206-05-8; BOC-Asp(OBzl)-OH, 7536-58-5; BOC-Leu-OH, 13139-15-6; H-Phe-NH_2, 5241-58-7; gastrin, 9002-76-0.

Supplementary Material Available: Tables of ^{1}H NMR data and assignments for compounds 1–17 (11 pages). Ordering information is given on any current masthead page.

3-Aminopyridazine Derivatives with Atypical Antidepressant, Serotonergic, and Dopaminergic Activities

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Minaprine [3-[(β-morpholinoethyl)amino]-4-methyl-6-phenylpyridazine dihydrochloride] is active in most animal models of depression and exhibits in vivo a dual dopaminomimetic and serotoninomimetic activity profile. In an attempt to dissociate these two effects and to characterize the responsible structural requirements, a series of 47 diversely substituted analogues of minaprine were synthesized and tested for their potential antidepressant, serotonergic, and dopaminergic activities. The structure–activity relationships show that dopaminergic and serotonergic activities can be dissociated. Serotonergic activity appears to be correlated mainly with the substituent in the 4-position of the pyridazine ring whereas the dopaminergic activity appears to be dependent on the presence, or in the formation, of a para-hydroxylated aryl ring in the 6-position of the pyridazine ring.

Minaprine [3-[(β-morpholinoethyl)amino]-4-methyl-6-phenylpyridazine dihydrochloride, 1] is a psychotropic drug that we synthesized[1] in continuation of our research on pyridazine derivatives.[2-10] In rodents, minaprine is active in most animal models of depression, is devoid of anticholinergic activity, and does not modify locomotor activity.[11-13] In humans, minaprine is well tolerated and has been shown to be more effective than placebo and equally efficient to maprotiline and nomifensine in the treatment of depressive disorders.[14-18] Minaprine is presently being extensively investigated as an antidepressant in most European countries and in the United States.

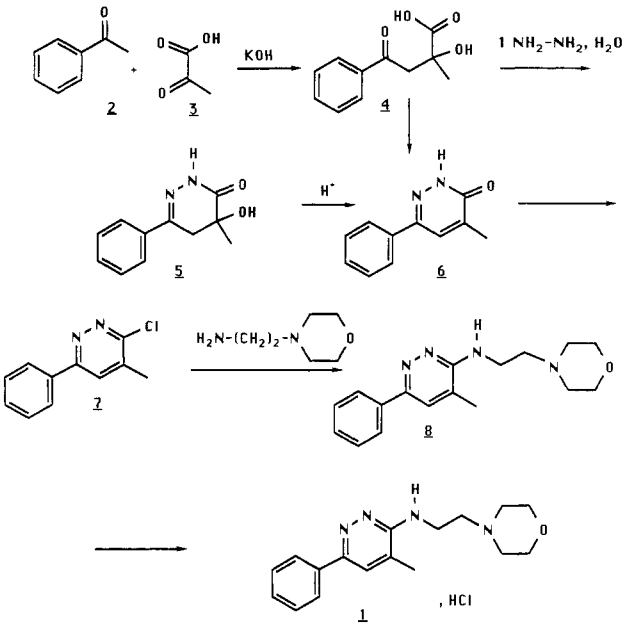
Biochemical and pharmacological studies show that in vivo minaprine enhances both serotonergic and dopaminergic transmission, but does not affect noradrenergic transmission.[11,19-22] In vitro, however, minaprine does not affect the uptake, the release, or the metabolism of serotonin and dopamine and does not interact with serotonin or dopamine receptors.[13,19-21] Thus, the mechanism(s) through which minaprine exhibits this dual serotoninomimetic and dopaminomimetic effect remain(s) unclear. Another question also arises, which is to investigate whether or not it is possible to dissociate these two effects by modifying the chemical structure of minaprine, and which are the structural requirements for each one of these effects.

In an attempt to approach these problems, we synthesized series of analogues of minaprine that were tested for their potential antidepressant, serotonergic, and dopaminergic activities. The main modifications concerned the basic side chain, the substituents in the 4-position of the pyridazine ring, and the exploration of the role and substitutions of the phenyl ring in the 6-position.

Chemistry

The two dominant aspects that characterized the synthesis of 3-[(aminoalkyl)amino]pyridazines were the creation of the pyridazinone ring, properly substituted in positions 4 and 6, and the subsequent branching of the basic side chain. The original synthesis of minaprine

Scheme I. Synthesis of Minaprine, a Generalizable Example for the Synthesis of 4,6-Disubstituted Analogues



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‡ Sanofi Recherche.
§ Present address: F. Hoffmann-La Roche and Co. Ltd., CH 4002 Basel, Switzerland.

(1) Wermuth, C. G.; Exinger, A. *Agressologie* **1972**, *13*, 285.
(2) Wermuth, C. G. *Agressologie* **1965**, *6*, 383.
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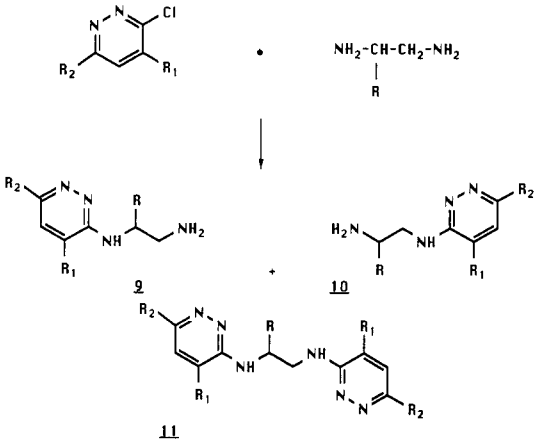
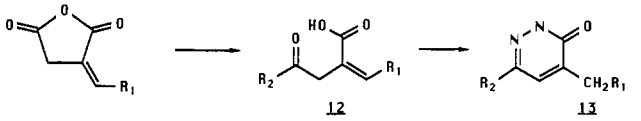
Scheme II. Synthesis of Minaprine Analogues Starting from Primary Diamines



Scheme III. Alternative Synthesis of Pyridazinones Starting with Itaconic Anhydride



(method A)[1-3] was applicable to a great number of 4,6-disubstituted analogues and is outlined in Scheme I.

The first step of this synthesis involves a ketolization reaction between acetophenone (**2**) and pyruvic acid (**3**) to yield α-methyl-β-benzoyllactic acid (**4**). As observed earlier, pyruvic acid can be replaced by its homologues,[23] and such ketolizations are applicable to aliphatic[24] or alicyclic[25-27] ketones. The cyclocondensation of the γ-keto acid with hydrazine led to the hydroxydihydropyridazinone **5** or its analogues when the reaction was performed under very mild conditions and when a strictly stoichiometric quantity of hydrazine was used.[24-27] The tertiary alcoholic group of **5** was then easily dehydrated in an acidic medium, yielding the pyridazinones of type **6**. However, these compounds could be directly obtained by treatment of the ketolic acids **4** with 1.5 equiv of hydrazine, the excess of hydrazine ensuring the basic dehydration of the intermediate hydroxydihydropyridazinone **5**.[28] The last steps of the synthesis are the conversion of the pyridazinones **6** into the imino chlorides **7** by treatment with phosphorus oxychloride and the nucleophilic displacement of the chlorine atom by the appropriate primary amine. The reactivity of the imino chlorides **7** toward nucleophilic attack is diminished by the adverse polarization of the adjacent C_6–N_1 imino function and drastic conditions are sometimes necessary.[29] We found that addition of a stoichiometric amount of ammonium chloride (or of the hydrochloride of the primary amine needed for the nucleophilic substitution) allowed easier operating conditions and gave rise to compound **8** and its analogues in high yields. This can be explained by a temporary complexation of the imino chloride that facilitates the nucleophilic attack.[30]

In the case of substitutions involving primary diamines (ethylene-, propylene-, or butylenediamine, α-methylethylenediamine), the expected aminopyridazines **9** and **10** were always accompanied by the symmetrically disubstituted diamines **11** (Scheme II). In addition, the substitution using α-methylethylenediamine gave rise to a 2:1 mixture of regioisomers **9** and **10**, the dominant compound **10** being substituted at the less hindered amino function (Scheme II).

We developed an alternative route for the synthesis of various pyridazinones involving the cyclocondensation of already unsaturated γ-keto acids.[31] This reaction took place with a shift of the α-methylene double bond into the pyridazinone ring **13** (Scheme III). The starting 2-alkylidene-4-oxo-4-arylbutanoic acids **12** were prepared either by a Friedel–Crafts acylation of benzene (or a substituted benzene) with itaconic anhydride[32] or by ring opening of α-alkylidene-β-butenolides.[33]

The syntheses illustrated in Scheme IV were utilized to introduce functional groups such as cyano, carbethoxy, or carboxamido in the 4-position of the pyridazinones. The key intermediate was the 4-carbethoxy-6-phenylpyridazinone **15**, which was prepared by alkylation of diethyl malonate with phenacyl chloride, cyclization of the keto diester **14** with hydrazine, and creation of the 4,5 double bond through bromine–acetic acid oxidation. Compound **15** was converted to its imino chloride **16** with use of phosphorus oxychloride. Ammonolysis of **16** yielded the carboxamido intermediate **18**. The 4-cyano derivative was prepared from **15** by successive ammonolysis and phosphorus oxychloride treatment, the latter ensuring both the dehydration of the carboxamide function and the conversion of the pyridazinone into its imino chloride **21**. The 3-chloro compounds **16**, **18**, and **21** were reacted with morpholinoethylamine as described above to give the analogues **17**, **19**, and **22**. Various 3-[(aminoalkyl)amino]pyridazines were prepared by these methods and are listed in Table I.

Pharmacological Results and Discussion

The 3-amino-6-phenylpyridazine derivatives described in this study were evaluated in mice for possible antidepressant, serotoninomimetic and dopaminomimetic ac-

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Table I. Synthetic Methods and Physical Data for Minaprine Analogues and Key Intermediates

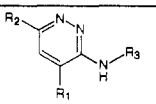
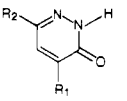
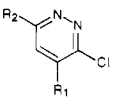
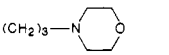
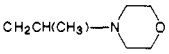
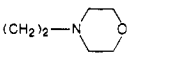
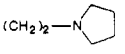
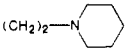
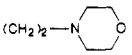
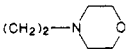
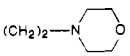
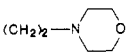
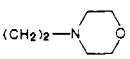
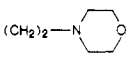
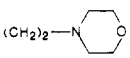
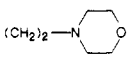
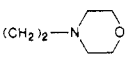
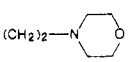
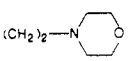
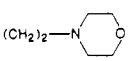
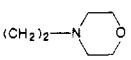
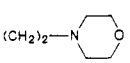
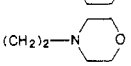
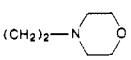
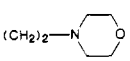
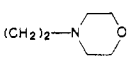
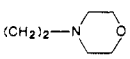
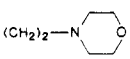
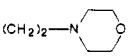
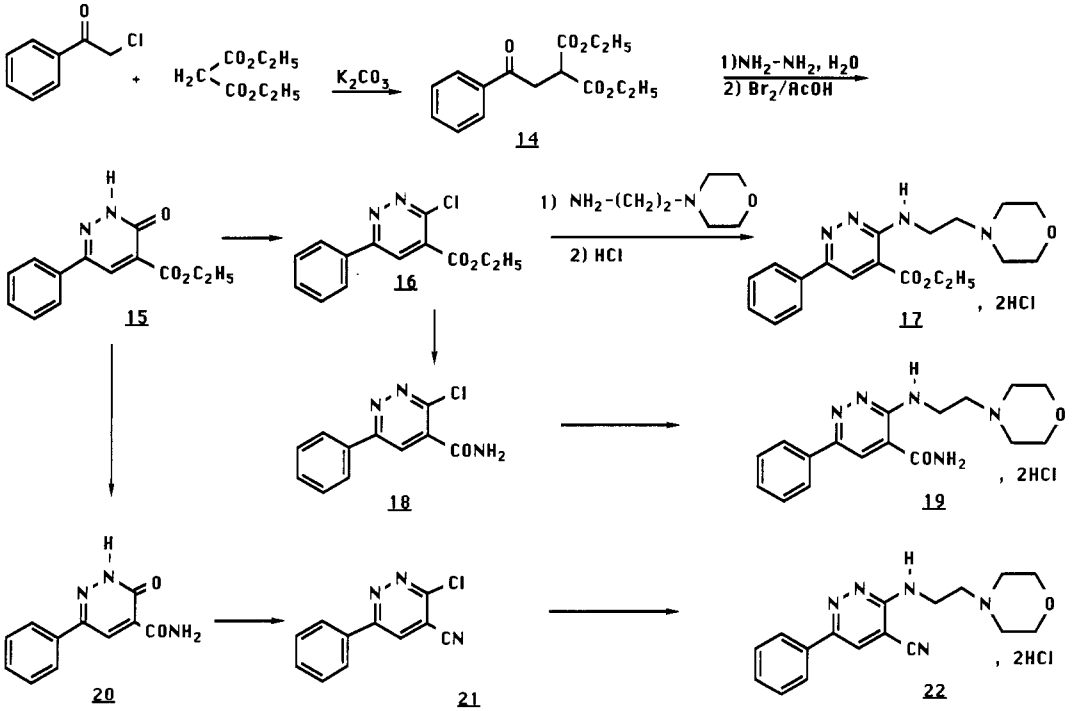
											
no.	R_1	R_2	R_3	mp, °C	formula	anal. (^{1}H NMR)	no.	method	mp, °C	no.	mp, °C
23	CH_3	C_6H_5		196	$C_{18}H_{24}N_4O\cdot2HCl$	CHN	**6**	A	(1)[c]	**7**	(1)
24	CH_3	C_6H_5		196	$C_{18}H_{24}N_4O\cdot HCl$	CHN	**6**	A	(1)	**7**	(1)
25	CH_3	C_6H_5		173	$C_{18}H_{24}N_4O\cdot2HCl$	CHN	**6**	A	(1)	**7**	(1)
26	CH_3	C_6H_5	$(CH_2)_2NH_2$	230	$C_{13}H_{16}N_4\cdot2HCl\cdot{}^1/_2H_2O$	CHN	**6**	A	(1)	**7**	(1)
27	CH_3	C_6H_5	$(CH_2)_3NH_2$	160	$C_{14}H_{18}N_4\cdot2HCl\cdot H_2O$	CHN	**6**	A	(1)	**7**	(1)
28[a]	CH_3	C_6H_5	$CH(CH_3)CH_2NH_2$	271	$C_{14}H_{18}N_4\cdot2HCl\cdot{}^1/_2H_2O$	CHN	**6**	A	(1)	**7**	(1)
	CH_3	C_6H_5	$CH_2CH(CH_3)NH_2$				**6**	A	(1)	**7**	(1)
29	CH_3	C_6H_5	$(CH_2)_4NH_2$	170 dec	$C_{15}H_{20}N_4\cdot2HCl\cdot H_2O$	CHN	**6**	A	(1)	**7**	(1)
30	CH_3	C_6H_5	$(CH_2)_2NHCH_3$	252	$C_{14}H_{18}N_4\cdot2HCl\cdot{}^1/_2H_2O$	CHN	**6**	A	(1)	**7**	(1)
31	CH_3	C_6H_5	$(CH_2)_2N(CH_3)_2$	238 dec	$C_{15}H_{20}N_4\cdot2HCl$	CHN	**6**	A	(1)	**7**	(1)
32	CH_3	C_6H_5	$(CH_2)_2N(C_2H_5)_2$	120	$C_{17}H_{24}N_4\cdot2$ oxalate	CHN	**6**	A	(1)	**7**	(1)
33	CH_3	C_6H_5	$(CH_2)_2N(nC_3H_7)_2$	168	$C_{19}H_{28}N_4\cdot2$ oxalate	CHN	**6**	A	(1)	**7**	(1)
34	CH_3	C_6H_5		148	$C_{17}H_{22}N_4\cdot2HCl$	CHN	**6**	A	(1)	**7**	(1)
35	CH_3	C_6H_5		246	$C_{18}H_{24}N_4\cdot2HCl$	CHN	**6**	A	(1)	**7**	(1)
36	H	C_6H_5		231	$C_{16}H_{20}N_4O\cdot2HCl$	CHN		A	(37)		(42)
37	C_2H_5	C_6H_5		172	$C_{18}H_{24}N_4O\cdot2HCl\cdot2H_2O$	CHN		A	(40)		(40)
22	CN	C_6H_5		144	$C_{17}H_{19}N_5O\cdot2HCl\cdot2H_2O$	CHN				**21**	206
19	$CONH_2$	C_6H_5		220	$C_{17}H_{21}N_5O\cdot2HCl$	CHN	**20**	*b*	300	**18**	204
17	$CO_2C_2H_5$	C_6H_5		148	$C_{19}H_{24}N_4O_3\cdot2HCl$	CHN	**15**	*b*	150	**16**	60
38	C_6H_5	C_6H_5		214	$C_{22}H_{24}N_4O\cdot2HCl$	CHN					
39	$CH_2C_6H_5$	C_6H_5		207	$C_{23}H_{26}N_4O\cdot2HCl$	CHN	**66**	B	190	**93**	116
40	CH_2-4′-Cl-C_6H_4	C_6H_5		162	$C_{23}H_{25}N_4OCl\cdot2HCl$	CHN	**67**	B	179	**94**	151
41	CH_2-2′-Cl-C_6H_4	C_6H_5		213	$C_{23}H_{25}N_4OCl\cdot2HCl$	CHN	**68**	B	150	**95**	135
42	CH_2-β-naphthyl	C_6H_5		220 dec	$C_{27}H_{28}N_4O\cdot2HCl\cdot2H_2O$	CHN	**69**	B	194	**96**	124
43	CH_3	4′-Cl-C_6H_4			$C_{17}H_{21}N_4OCl\cdot2HCl$	CHN	**70**	A		**97**	
44	CH_3	3′-Cl-C_6H_4		214	$C_{17}H_{21}N_4OCl\cdot2HCl$	CHN	**71**	A	(40)	**98**	112
45	CH_3	2′-Cl-C_6H_4		219	$C_{17}H_{21}N_4OCl\cdot2HCl$	CHN	**72**	A	232	**99**	112
46	CH_3	4′-F-C_6H_4		231	$C_{17}H_{21}N_4OF\cdot2HCl$	CHN	**73**	A	234	**100**	178
47	CH_3	2′-F-C_6H_4		230	$C_{17}H_{21}N_4OF\cdot2HCl$	CHN	**74**	A	196	**101**	105
48	CH_3	4′-CH_3-C_6H_4			$C_{18}H_{24}N_4O\cdot2HCl$	CHN	**75**	A	(40)	**102**	(42)
49	CH_3	3′-CH_3-C_6H_4		177	$C_{18}H_{24}N_4O\cdot2HCl$	CHN	**76**	A	235	**103**	116
50	CH_3	2′-CH_3-C_6H_4		189	$C_{18}H_{24}N_4O\cdot2HCl$	CHN	**77**	A	185	**104**	175
51	CH_3	4′-OCH_3-C_6H_4		162	$C_{18}H_{24}N_4O_2\cdot2HBr$	CHN	**78**	A	228	**105**	115
52	CH_3	3′-OCH_3-C_6H_4		220	$C_{18}H_{24}N_4O_2\cdot2HCl$	CHN	**79**	A	188	**106**	88
53	CH_3	2′-OCH_3-C_6H_4		224	$C_{18}H_{24}N_4O_2\cdot2HCl$	CHN	**80**	A	210	**107**	96

Table I (Continued)

no.	R_1	R_2	R_3	mp, °C	formula	anal. (^{1}H NMR)	no.	method	mp, °C	no.	mp, °C
54	CH_3	4′-NH_2-C_6H_4	$(CH_2)_2$-N(morpholine)		$C_{17}H_{23}N_5O\cdot 2HCl\cdot H_2O$	CHN	**81**	*b*		**108**	*b*
55	CH_3	4′-NO_2-C_6H_4	$(CH_2)_2$-N(morpholine)		$C_{17}H_{21}N_5O_3\cdot 2HCl\cdot H_2O$	CHN	**82**	A	(42)	**109**	(42)
56	CH_3	4′-OH-C_6H_4	$(CH_2)_2$-N(morpholine)	170	$C_{17}H_{22}N_4O_2\cdot 2HBr$	CHN	**83**	*b*	(40)	**110**	*b*
57	CH_3	3′-OH-C_6H_4	$(CH_2)_2$-N(morpholine)		$C_{17}H_{22}N_4O_2\cdot 2HBr$	CHN	**84**	*b*		**111**	*b*
58	CH_3	2′-OH-C_6H_4	$(CH_2)_2$-N(morpholine)	162	$C_{17}H_{22}N_4O_2\cdot 2HBr$	CHN	**85**	*b*		**112**	*b*
59	CH_3	CH_3	$(CH_2)_2$-N(morpholine)	278	$C_{12}H_{20}N_4O\cdot 2HCl$	CHN	**86**	A	129	**113**	
60	CH_3	c-C_6H_{11}	$(CH_2)_2$-N(morpholine)	237	$C_{17}H_{28}N_4O\cdot 2HCl$	CHN	**87**	A	173	**114**	
61	CH_3	$CH_2C_6H_5$	$(CH_2)_2$-N(morpholine)	160	$C_{18}H_{24}N_4O\cdot 2HCl$	CHN	**88**	A	160	**115**	300
62	CH_3	α-naphthyl	$(CH_2)_2$-N(morpholine)	200	$C_{21}H_{24}N_4O\cdot 2HCl$	CHN	**89**	A	(41)	**116**	(42)
63	CH_3	β-naphthyl	$(CH_2)_2$-N(morpholine)	200	$C_{21}H_{24}N_4O\cdot 2HCl$	CHN	**90**	A		**117**	
64	CH_3	2′-thienyl	$(CH_2)_2$-N(morpholine)	234	$C_{15}H_{20}N_4OS\cdot 2HCl$	CHN	**91**	A	236	**118**	146
65	CH_3	3′-thienyl	$(CH_2)_2$-N(morpholine)	152	$C_{15}H_{20}N_4OS\cdot 2HCl$	CHN	**92**	A	246	**119**	171

[a] 3:1 mixture. [b] See special procedures. [c] Numbers in parentheses refer to reference numbers.

Scheme IV. Synthesis of 4-Cyano, 4-Aminocarbonyl, and 4-Carbethoxy Analogues of Minaprine



tivities. The antagonism of reserpine-induced ptosis was used to screen for antidepressant activity.[34] The potentiation of 5-hydroxytryptophan (5-HTP) was taken as an index of serotonergic activity.[35] The decrease of the turning behavior induced by a unilateral striatal 6-hydroxydopamine lesion was considered as predictive of dopaminergic activity.[36] Minaprine and its analogues were

(34) Gouret, C.; Mocquet, G.; Coston, A.; Raynaud, G. *J. Pharmacol.* **1977**, *8*, 333.

(35) Lessin, A. W. *Biochem. Pharmacol.* **1973**, *2*, 290.

(36) Von Voigtlander, P. F.; Moore, K. E. *Neuropharmacology* **1973**, *12*, 451.

Table II. Pharmacological Data for Reference Compounds

	reserpine ptosis: ED_{50}, mg/kg ip	potentiation 5-HTP: ED_{50}, mg/kg ip	turning: MED, mg/kg ip
imipramine	2.3 (2.2–2.4)	In.[b] (>30)	In. (>20)
chlorimipramine	3.3 (1.8–4.1)	4.8 (3.0–7.9)	NT
nomifensine	0.17 (0.13–0.22)	In. (>30)	5[c]
apomorphine	NT[a]	NT	0.05
(+)-amphetamine	NT	NT	2.5

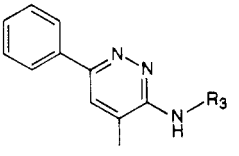
[a] Not tested.

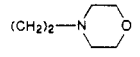
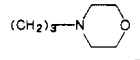
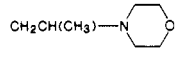
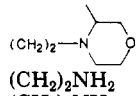
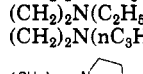
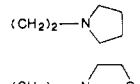
compared to five reference compounds: imipramine, chlorimipramine, nomifensine, apomorphine, and (+)-amphetamine. As shown in Table II, reserpine-induced ptosis was antagonised by the antidepressant drugs (ADDs) imipramine, chlorimipramine, and nomifensine; chlorimipramine was the only ADD to potentiate 5-HTP-induced tremor. The dopamine receptor agonist apomorphine decreased the spontaneous ipsilateral turning occurring in the lesioned mice, whereas nomifensine, a dopamine uptake inhibitor, and (+)-amphetamine, a dopamine releaser, both increased this behavior; imipramine was inactive in this latter model. Like the parent compound minaprine, most of the compounds tested induced seizures and were screened for antidepressant, serotoninomimetic, and dopaminomimetic activity at a dose not higher than 50% of the threshold dose inducing seizures. If a compound was found active at this dose, dose-response curves were generated, from which half-maximal effective values (ED_{50}) were calculated by log-probit analysis. For turning experiments, a minimal effective dose (MED; $p < 0.05$) was generated.

The structure–activity relationships in this series were examined by various types of structural changes. The nature of the side chain on the exocyclic amine was modified. The effects of substituents in the 4-position of the pyridazine ring were examined. The phenyl substituents were varied to test steric and electronic effects. Finally, the phenyl ring itself was replaced by other aromatic heteroaromatic and nonaromatic cycles.

As shown in Table III, lengthening the morpholinoethyl side chain to a 3-morpholinopropyl side chain led to compound **23**, which was inactive in all screening procedures but which still induced seizures with a potency comparable to that of the parent compound **1**. Replacing the morpholinoethyl side chain by a 2-morpholinopropyl side chain (**24**) or substituting the morpholine ring in the α position by a methyl (**25**) yielded compounds that were weak antagonists of reserpine-induced ptosis (antidepressant activity). They were inactive in the 5-HTP potentiation test and in the turning model (5-HT and DA activities). Again, for compounds **24** and **25**, convulsant potency was not significantly different from that of the parent compound **1**. Replacing the morpholinoethyl side chain with an aminoethyl (**26**), a 3-aminopropyl (**27**), a 2-aminopropyl (**28**), or a 4-aminobutyl (**29**) side chain led to compounds that were poor antagonists of reserpine-induced ptosis and that did not potentiate 5-HTP. Compounds **26**, **27**, and **29** exhibited fairly potent dopaminomimetic activity as predicted by the turning behavior model; compound **28** was poorly active. Interestingly these compounds with a primary amine side chain were considerably less convulsant than the parent compound **1**. The monomethyl (**30**) and the dimethyl (**31**) derivatives were weak antagonists of reserpine-induced ptosis, were inactive in potentiating 5-HTP, and did not modify turning behavior. These compounds were weaker convulsants than compound **1**. The diethylamino (**32**), dipropylamino (**33**), pyrrolidino (**34**), and piperidino (**35**) derivatives were inactive in all screening procedures and were as potent as compound **1** in eliciting seizures. The results obtained by altering the morpholinoethyl side chain of the parent compound **1**

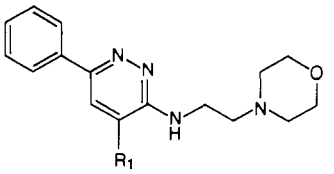
Table III. Pharmacological Results: The Effects of the Nature of the Amino Side Chain



no.	R_3	acute toxicity: LD_{50}, mg/kg ip	reserpine ptosis: ED_{50}, mg/kg ip	potentiation 5-HTP: ED_{50}, mg/kg ip	turning: MED, mg/kg ip
1		63	5 (4–7)	24 (19–31)	0.06
23		66	>30	>25	2
24		54	13 (11–16)	>15	2
25		110	20 (11–34)	30[b]	>2
26	$(CH_2)_2NH_2$	300	16 (14–18)	>30	0.5
27	$(CH_2)_3NH_2$	300	50[b]	>30	0.1
28	$CH(CH_3)CH_2NH_2$ $CH_2CH(CH_3)NH_2$	>200	34 (31–38)	>30	2
29	$(CH_2)_4NH_2$	300	>50	>30	0.1
30	$(CH_2)_2NHCH_3$	>200	36 (24–55)	≥30	2
31	$(CH_2)_2N(CH_3)_2$	118	29 (27–31)	>30	10
32	$(CH_2)_2N(C_2H_5)_2$	80	>30	>30	>10
33	$(CH_2)_2N(nC_3H_7)_2$	76	>30	>30	2
34		70	>30	NT[a]	NT[a]
35		37	≥10	>10	2

[a] NT: not tested. [b] 50% activity at this maximal tested dose.

Table IV. Pharmacological Results for 4-Substituted Analogues of Minaprine



no.	R_1	acute toxicity: LD_{50}, mg/kg ip	reserpine ptosis: ED_{50}, mg/kg ip	potentiation 5-HTP: ED_{50}, mg/kg ip	turning: MED, mg/kg ip
1	CH_3	63	5.0 (4–7)	24 (19–31)	0.06
36	H	250	6.0 (5.9–6.7)	3.7 (3.0–4.0)	0.5
37	C_2H_5	31	>10	>10	2
22	CN	>250	6.6 (5.9–7.3)	5.0 (4.4–5.7)	0.1
19	$CONH_2$	NT[a]	>40	>60	2
17	$CO_2C_2H_5$	180	>50	NT	0.5
38	C_6H_5	150	>50	>25	>2
39	$CH_2C_6H_5$	131	>50	>50	2
40	CH_2-4′-Cl-C_6H_4	180	>30	>30	10
41	CH_2-2′-Cl-C_6H_4	95	>50	>50	10
42	CH_2-β-naphthyl	275	>50	>100	2

[a] NT: not tested.

indicate that antidepressant, serotoninomimetic, and dopaminomimetic activities are not necessarily correlated to convulsant activity. Moreover, the primary amine derivatives **26–29** were fairly potent in the turning model and did not potentiate 5-HTP-induced tremor, suggesting that dopaminomimetic activity can be dissociated from serotoninomimetic activity. As far as overall activity is concerned, the parent compound **1** was clearly the most potent compound, emphasizing the importance of the morpholino group for activity.

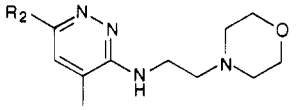
The effects of substituents in the 4-position of the pyridazine ring are shown in Table IV. Suppressing (**36**) or replacing the methyl in the 4-position by a cyano group (**22**) led to compounds that were as active as **1** in antagonizing reserpine-induced ptosis, more active in potentiating 5-HTP-induced tremors, and slightly less active in the turning model; moreover, these compounds were weaker convulsants than **1**. All the other substitutions in the 4-position yielded compounds that were inactive, with the exception of compound **17**, which exhibited moderate dopaminomimetic activity. Thus the nature of the substituent in the 4-position is crucial for activity. Electronic factors are not involved since the 4-methyl-substituted compound **1** and the 4-cyano-substituted compound **22** exhibit comparable activities. Steric factors may be involved since more bulky substituents such as an ethyl in **37** or a phenyl in **38** in the 4-position led to inactive compounds. Another interpretation of these results could be that a readily metabolizable group in the 4-position is necessary for antidepressant, serotoninomimetic, and dopaminomimetic activities; one could hypothesize that the activities of compounds **1** and **22** could be due to their metabolization to the nonsubstituted derivative **36**. In view of this hypothesis the virtual inactivity of compounds **17** and **19** is surprising since one would expect these compounds to be hydrolyzed to the corresponding 4-carboxylic derivative, which would yield compound **36** by decarboxylation. However, one cannot preclude simultaneous inactivating metabolic attacks of compounds **17** and **19** on the phenyl ring or on the (morpholinoethyl)amino side chain.

If we consider the convulsant activity, it can be seen that the nature of the substituent in the 4-position of the pyridazine ring also markedly affected this potency. Electronic, and not steric, factors seem to be involved for this activity. Substitution in the 4-position with electron-donating groups (**1**, **37**) increased convulsant activity, whereas substitution by electron-attracting groups **22** led to compounds that were weak convulsants. Steric factors in this position do not seem to affect convulsant activity, since the 4-methyl derivative **1** was considerably more potent than the 4-cyano derivative **22** in inducing seizures and derivatives with bulky substituents in the 4-position (**38–42**) were as weak as the unsubstituted compound **36** in inducing seizures.

The effects of various substituents on the phenyl ring in the 6-position in modifying the activity of the parent compound **1** is shown in Table V. Practically all the substitutions led to loss of activity with the exception of the 2′-hydroxy (**58**), 3′-methoxy (**52**), 4′-chloro (**43**), and 4′-fluoro (**46**) derivatives, which were mainly active in the reserpine and in the turning models, and the 4′-hydroxy (**56**) derivative, which exhibited selective dopaminomimetic activity. Interestingly the 4′-chloro and 4′-fluoro derivatives were poorly active in the turning model, although they were active in the other screening procedures, suggesting that para-hydroxylation is necessary for dopaminomimetic activity. The apparent loss of activity entailed by most of the substitutions on the phenyl ring should be interpreted with caution since, with the exception of the hydroxylated derivatives **56–58**, the convulsant potency of these compounds was at best equal to that of the parent compound **1** and usually much greater. For this reason most of the compounds could not be tested at doses higher than 1–10 mg/kg, doses at which they were found inactive. With respect to the convulsant potency of these compounds, the 2′-substituted compounds were more potent than the corresponding 3′-substituted derivatives, which in turn were more convulsant than the 4′-substituted derivatives. Steric factors are probably involved since the 4′-chloro and the 4′-methyl derivatives are equipotent in inducing seizures. The relatively low convulsant potency of the hydroxylated derivatives is difficult to interpret since several factors may be involved such as metabolic degradation or low penetration into the brain.

Replacing the phenyl ring by a methyl group yielded a totally inactive compound **59**, and replacing the phenyl ring by a cycloalkyl or other aromatic rings led to a dissociation between serotoninergic and dopaminergic activities. Thus the cyclohexyl (**60**) and the α- and β-thienyl derivatives (**64**, **65**) were active as antidepressants and serotoninomimetics, whereas the α-naphthyl derivative (**62**) was selectively dopaminergic. The inactivity of the cyclohexyl and the α- and β-thienyl derivatives in the turning

Table V. Pharmacological Results: The Effects of the Aromatic Substitution in the Position 6



no.	R_2	acute toxicity: LD_{50}, mg/kg ip	reserpine ptosis: ED_{50}, mg/kg ip	potentiation 5-HTP: ED_{50}, mg/kg ip	turning: MED, mg/kg ip
1	C_6H_5	63	5 (4–7)	24 (19–31)	0.06
43	4′-Cl-C_6H_4	62	>10	5.8 (4.9–6.7)	>2
44	3′-Cl-C_6H_4	21	>10	>10	>2
45	2′-Cl-C_6H_4	7	>5	NT[c]	1
46	4′-F-C_6H_4	45	4.5 (1.9–10.7)	4.7 (1.9–11.7)	2
47	2′-F-C_6H_4	26	>10	>10	0.5
48	4′-CH_3-C_6H_4	51	>15	>15	0.5
49	3′-CH_3-C_6H_4	23	10[a]	>10	>2
50	2′-CH_3-C_6H_4	5	>1	>1	>1
51	4′-OCH_3-C_6H_4	19	>5	>5	>2
52	3′-OCH_3-C_6H_4	18	6 (5–9)	>5	0.5
53	2′-OCH_3-C_6H_4	40	>10	>10	0.5
54	4′-NH_2-C_6H_4	15	>5	NT	>2
55	4′-NO_2-C_6H_4	35	>10 (3.2–6.3)	>10	NT
56	4′-OH-C_6H_4[b]	>300	>40	>100	0.1
57	3′-OH-C_6H_4	>200	>50	>30	10
58	2′-OH-C_6H_4	>200	16 (16–20)	17 (17–20)	0.1
59	CH_3	>100	>50	>50	>10
60	c-C_6H_{11}	43	4.5 (3.2–6.3)	16 (3.0–38)	>10
61	CH_2-C_6H_5	30	>10	>10	2
62	α-naphthyl	7	>2.5	>2.5	0.01
63	β-naphthyl	23	>10	NT	>2
64	2′-thienyl	59	4.8 (4.0–5.7)	4.2 (3.8–4.9)	>10
65	3′-thienyl	72	10 (9.0–12.0)	1.7 (1.4–2.1)	>10

[a] 50% activity at this maximal tested dose. [b] See ref 46. [c] NT: not tested.

model could again be interpreted as indicating that para-hydroxylation is necessary for dopaminergic activity. The benzyl derivative **61** as well as the α- and β-naphthyl derivatives were more potent in inducing seizures than the parent compound **1**, suggesting again that increasing the bulk of the substituent in the 6-position increases convulsant potency.

The structure–activity relationships in this series show that serotonergic, dopaminergic, and convulsant activities can be dissociated. Notably, the fact that serotonergic and dopaminergic activities are not always correlated strongly suggests that the serotoninomimetic and dopaminomimetic activities of the parent compound minaprine are mediated by different mechanisms and/or different active metabolites. Thus structure–activity relationships in this series should be analyzed in terms of potential metabolism as well as in terms of absolute structures. Serotonergic activity appears to be correlated mainly with the nature of the substituent in the 4-position of the pyridazine ring, but is also influenced by the nature of the side chain in the 3-position of this ring. The dopaminergic activity appears to be mainly dependent on the possibility of a para-hydroxylation of the heteroaromatic substituent in the 6-position of the pyridazine ring, although the nature of the side chain in the 3-position and of the substituent in the 4-position are also involved. Finally, the interpretation of the structure–activity relationships of the convulsant potency of the compounds in this series is complex, since altering either the side chain in the 3-position or the nature of the substituents in the 4- or in the 6-position modifies seizure threshold.

Experimental Section

Chemical Methods. Melting points were obtained on a calibrated Kofler hot stage apparatus and are uncorrected. IR spectra were measured with a Beckman Acculab-4 spectrophotometer, and ^1H NMR spectra were recorded on a Bruker WP-60 spectrometer using the δ scale with reference to Me_4Si for $CDCl_3$ solutions. Chemical shifts were as expected and are only described for some selected compounds.

Some 3(2*H*)-pyridazinones are described in the literature. These are 4-methyl-6-phenyl-3(2*H*)-pyridazinone,[1] 6-phenyl-3-(2*H*)-pyridazinone,[37] 4,6-diphenyl-3(2*H*)-pyridazinone,[38,39] 6-(*p*-hydroxyphenyl)-, 6-(*m*-chlorophenyl)-, 6-(*o*-chlorophenyl)-, 6-(*p*-methylphenyl)-, and 6-(*p*-methoxyphenyl)-4-methyl-3(2*H*)-pyridazinones,[40] 6-α-naphthyl-4-methyl-3(2*H*)-pyridazinone,[41] and 6-(*p*-nitrophenyl)-4-methyl-3(2*H*)-pyridazinone.[42] The corresponding 3-chloropyridazines have been prepared to synthesize GABA antagonists.[42]

Most of the new 3(2*H*)-pyridazinones were prepared by using methods A–C. A typical example for each method is given below.

Method A (Scheme I). 6-(3-Thienyl)-4-methyl-3(2*H*)-pyridazinone (92). A 2-L Erlenmeyer flask containing 70.4 g (0.8 mol) of pyruvic acid was cooled in an ice bath and the acid neutralized (phenolphthalein) with 20% KOH solution with stirring. 3-Acetylthiophene (0.8 mol) was added followed by a solution of 56 g of KOH pellets in 800 mL of methanol. The homogeneous mixture was kept at 4 °C for 96 h. The solution was then chilled in an ice bath and neutralized (phenolphthalein) with 10 N H_2SO_4, and the methanol was removed under reduced pressure. The residual mixture was acidified with 10 N H_2SO_4, diluted with water in order to dissolve the precipitated K_2SO_4, and extracted with three portions of diethyl ether. The combined ethereal layers were washed with brine and extracted with 400 mL of 10% $KHCO_3$. The basic solution was washed with a small amount of ether and acidified with 10 N H_2SO_4. The crude keto acid precipitated and was recrystallized in water (or in benzene). Pure 3-(3-thenoyl)-2-hydroxy-2-methylpropionic acid was obtained (25.5 g, 12%): ^1H NMR ($CDCl_3$) δ 1.55 (s, 3 H, H_3CCCH_2), 3.46

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(C_{AB}, J_{AB} = 17.3 Hz, δ_A = 3.37, δ_B = 3.65, 2 H, CH_2CCH_3), 7.34 (dd, J = 5.3, J = 3.0 Hz, 1 H, H_4 thienyl), 7.54 (dd, J = 3.0, J = 1.5 Hz, 1 H, H_2 thienyl).

The unreacted ketone can be recovered from the ethereal layer by evaporation and recrystallization (50 g).

To a solution of 25.3 g (0.12 mol) of 3-(3-thenoyl)-2-hydroxy-2-methylpropionic acid in 80 mL of 1-butanol contained in a Claisen flask was added 8.9 g (0.18 mol) of hydrazine hydrate. The water–butanol azeotrope was slowly distilled, followed by about 50 mL of pure butanol. After cooling, the expected 3-(2*H*)-pyridazinone crystallized. It was recrystallized in acetic acid, yielding 18 g (75%) of the title compound: mp 246 °C; ^{1}H NMR (DMSO-d_6) δ 1.98 (d, J = 0.7 Hz, 3 H, H_3CC=CH), 7.3–7.6 (m, 2 H, H_5 thienyl), 7.76 (q, J = 0.7 Hz, 1 H, HC=CH_3), 7.8–8.0 (m, 1 H, H_2 thienyl), 12.78 (s, 1 H, HNC=O). Anal. C, H, N.

By the same procedure and starting from the appropriate methyl ketones, 3(2*H*)-pyridazinones **70**, **73**, **74**, **76**, **77**, **79**, **80**, **86**, **87**, **88**, **90**, **91**, and **92** were prepared (Table I).

Method B (Scheme III). 4-(2-Chlorobenzylidene)-6-phenyl-3(2*H*)-pyridazinone (68). (a) α-(2-Chlorobenzylidene)-γ-phenyl-β,γ-butenolide (120). A mixture of 53.5 g (0.3 mol) of β-benzoylpropionic acid, 33.6 mL (0.3 mol) of 2-chlorobenzaldehyde, 24.6 g (0.3 mol) of freshly fused sodium acetate, and 170 mL of acetic anhydride was maintained at 100 °C for 2 h. The hot solution was decanted from the sodium acetate, about 40 mL of 95% EtOH was added, and the mixture was kept at 4 °C for 3 h. The crystals were collected and washed first with a small amount of cold 95% ethanol followed by 200 mL of boiling water to remove any sodium acetate present. After drying, 59.6 g (71%) of the butenolide was obtained as a yellow solid which was pure enough for the following step. An analytical sample (mp 203.5 °C) could be obtained by recrystallization in AcOEt.

(b) 2-(2-Chlorobenzylidene)-3-benzoylpropionic Acid.[33] A mixture of 29.8 g (0.105 mol) of butenolide **120**, 105 mL of 2 N NaOH, and 200 mL of 95% ethanol was refluxed for 1 h. After this time, the ethanol was removed, and the residue was diluted by means of 150 mL of water and acidified (pH = 1) with 10% HCl. The precipitated keto acid was collected, washed with water, dried, and recrystallized in 95% ethanol: yield 25 g (79%); mp 146 °C.

(c) 4-(2-Chlorobenzylidene)-6-phenyl-3(2*H*)-pyridazinone (68). 2-(2-Chlorobenzylidene)-3-benzoylpropionic acid (**13**) was cyclized with hydrazine hydrate as reported in method A (mp 150 °C).

Starting from the appropriate aldehydes, four new 3(2*H*)-pyridazinones (**66–69**) were prepared by using the same procedures (Table I).

General Procedure for 3-Chloropyridazines. A mixture containing the appropriate substituted 3(2*H*)-pyridazinone and phosphorus oxychloride (2 mL for 1 mmol) was heated at 80 °C for 2–6 h. After reduction of the excess volume of $POCl_3$ by distillation under reduced pressure, the mixture was carefully poured on crushed ice. The water was rendered alkaline (20% NaOH). Crude 3-chloropyridazine was collected by filtration, washed with water, and dried under vacuum. The crude product was purified by chromatography on silica gel with a mixture of hexane–ethyl acetate as eluent or by recrystallization in 2-propanol or methanol.

Most of the new 3-chloropyridazines were prepared by using this procedure. Data are given in Table I.

General Procedures for 3-[(β-Morpholinoethyl)amino]-pyridazines. Two methods (C, D) were used to prepare this compound.

Method C. A mixture of substituted 3-chloropyridazine (0.01 mol), an excess of the appropriate amine (0.05 mol), and 1 equiv of the corresponding amine hydrochloride (or ammonium chloride) was heated at 170 °C under argon with magnetic stirring for 1–1.5 h. The excess of amine was removed under reduced pressure. The residue was extracted with ethyl acetate, washed with water and brine, and dried over $MgSO_4$. After solvent evaporation, the product was crystallized and recrystallized with a mixture of ethyl ether–methanol.

Method D. A solution of substituted 3-chloropyridazine (0.01 mol) and 4–6 equiv of the appropriate amine in 1-butanol (25 mL) was refluxed for 48 h. The solvent was evaporated under reduced pressure. The residue was then treated as described in method C.

Data are given in Table I.

The corresponding hydrochloride or hydrobromide were prepared by treating an ethereal solution of the base with gaseous hydrogen chloride or by adding 1 equiv of concentrated aqueous solution of hydrogen chloride or hydrogen bromide. Data are also given in Table I.

Special Procedures (Scheme IV). 4-(Ethoxycarbonyl)-6-phenyl-3(2*H*)-pyridazinone (15). (a) 4,5-Dihydro-4-(ethoxycarbonyl)-6-phenyl-3(2*H*)-pyridazinone. To a solution of 40.5 g (0.145 mol) of ethyl 2-carbethoxy-3-benzoylpropionate[43,44] in 70 mL of absolute ethanol cooled to 0 °C was added dropwise 7.3 g (0.145 mol) of hydrazine hydrate with stirring. The cooling bath was then removed and stirring was continued for 24 h. The expected dihydropyridazinone was then filtered off. An additional amount of 3.6 g (0.07 mol) of hydrazine hydrate was added to the filtrate, and after the mixture was allowed to stand for 24 h at room temperature, a second crop of the dihydropyridazinone was collected. The combined crops were purified either by recrystallization in ethanol or by column chromatography (silica gel, elution with 1:1 hexane–ethyl acetate). The yield was 21.5 g (60%) of a white powder: mp 158 °C; IR (KBr) 3310, 1740, 1675 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, J = 7.0, 3 H, OCH_2CH_3), 3.1–3.7 (m, 3 H, $CH_2CH(CO)_2$), 4.27 (q, J = 6.9, 2 H, OCH_2CH_3), 7.4–8.0 (m, 5 H, C_6H_5), 9.15 (br s, exchangeable with D_2O, 1 H, CON*H*).

(b) 4-(Ethoxycarbonyl)-6-phenyl-3(2*H*)-pyridazinone (15). To a solution of 9.0 g (0.036 mol) of dihydropyridazinone **121** in 200 mL of acetic acid was added dropwise a solution of 11.5 g (0.072 mol) of bromine in 50 mL of acetic acid. After complete reaction the solvent was evaporated under reduced pressure. To the residue was added 200 mL of water and the mixture was extracted with AcOEt. The collected organic layers were washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was recrystallized in isopropyl alcohol or purified by chromatography (silica gel, elution with 1:1 cyclohexane–AcOEt): yield 5.8 g (60%) of pale yellow needles; mp 150 °C; IR (KBr) 3010, 1720, 1670 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, J = 7.2 Hz, 3 H, OCH_2CH_3), 4.47 (q, J = 7.2 Hz, 2 H, OCH_2CH_3), 7.5–7.8 (m, 5 H, C_6H_5), 8.32 (s, 1 H). Anal. C, H, N.

4-(Aminocarbonyl)-6-phenyl-3(2*H*)-pyridazinone (20). A solution of 2 g (0.008 mol) of 4-(ethoxycarbonyl)-6-phenyl-3-(2*H*)-pyridazinone (**15**) in 40 mL of concentrated ammonia (33%) was kept at 20 °C overnight. The aminocarbonyl derivative precipitated as a white powder: mp 300 °C; yield 86%; IR (KBr) 3330–3140, 1710, 1690 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 7.4–8.2 (m, 5 H, C_6H_5), 8.50 (s, 1 H, HC=CC=O), 8.90 (broad s, exchangeable with D_2O, 2 H, CONH_2). Anal. C, H, N.

3-Chloro-4-cyano-6-phenylpyridazine (21). A solution of 1.5 g (0.007 mol) of 4-(aminocarbonyl)-6-phenyl-3(2*H*)-pyridazinone (**20**) in 15 mL of phosphorus oxychloride was heated at 80–90 °C for 3 h. The excess of phosphorus oxychloride was removed in vacuo, and 50 mL of iced water was added. The expected pyridazine precipitated and was recrystallized in 2-propanol: yield 60%; mp 206 °C; IR ($CHCl_3$) 2230 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.5–8.2 (m, 5 H, C_6H_5), 8.13 (s, 1 H, HCC=CCN).

3-Chloro-4-(aminocarbonyl)-6-phenylpyridazine (18). A solution of 1.5 g (0.006 mol) of 3-chloro-4-(ethoxycarbonyl)-6-phenylpyridazine (**16**) in 60 mL of concentrated ammonia was stirred overnight at room temperature. The product was filtered off and dried in vacuo: mp 204 °C; yield 84%; IR (KBr) 3300–3150, 1670 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 6.15 (br s, 2 H, exchangeable with D_2O, CONH_2), 7.6–8.0 (m, 5 H, C_6H_5), 8.41 (s, 1 H, HCC=CCONH_2).

3-[(β-Morpholinoethyl)amino]-4-methyl-6-(4-amino-

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phenyl)pyridazine (54). A solution of 0.3 g (0.0009 mol) of 3-[(morpholinoethyl)amino]-4-methyl-6-(4-nitrophenyl)pyridazine (**55**) in 70 mL of ethyl ether was saturated with gaseous hydrogen chloride. The mixture was hydrogenated at 2–4 atm over 10% Pd/C for 1 h. Filtration and evaporation to dryness gave 0.25 g (60%) of reduced product **54**: ^{1}H NMR (D_2O) δ 2.05 (br s, 3 H, H_3CC=CH), 3.2–3.6 (m, 6 H, $(CH_2)_3N$), 3.6–4.1 (m, 6 H, $O(CH_2)_2$ + $NHCH_2$), 6.8–8.4 (m, 6 H, C_6H_5 + HC=CCH_3). Anal. (hydrochloride, $1.5H_2O$) C, H, N.

3-[(β-Morpholinoethyl)amino]-4-methyl-6-(2-hydroxyphenyl)pyridazine (58), 3-[(β-Morpholinoethyl)amino]-4-methyl-6-(3-hydroxyphenyl)pyridazine (57), and 3-[(β-Morpholinoethyl)amino]-4-methyl-6-(4-hydroxyphenyl)pyridazine (56). These compounds were prepared by hydrolysis of the corresponding methoxyphenyl derivatives. The procedure is illustrated for compound **56**.

3-[(β-Morpholinoethyl)amino]-4-methyl-6-(4-methoxyphenyl)pyridazine (51) (19 g, 0.06 mol) was refluxed in 150 mL of a mixture of acetic acid (100 mL) and concentrated aqueous hydrogen bromide (50 mL) (or hydrogen chloride) for 6 h. The solvents were removed. The oil crystallized upon dissolution in ethyl alcohol and ether addition. It was recrystallized in ethyl alcohol: mp 162 °C; yield 78% (21.5 g); ^{1}H NMR (D_2O) δ 2.36 (br s, 3 H, H_3C=CH), 3.3–3.7 (m, 6 H, $(CH_2)_3N$), 3.8–4.2 (m, 6 H, $O(CH_2)_2$), 7.35 ($C_{(AB)2}$, J_{AB} = 8.0, σ = 0.67, 4 H, C_6H_4), 7.87 (br s, 1 H, HC=CCH_3). Anal. C, H, N.

3-[(Aminoethyl)amino]-4-methyl-6-phenylpyridazine (26). 3-Chloro-4-methyl-6-phenylpyridazine (**7**) (12.0 g, 0.058 mol) and a large excess of ethylenediamine (35 g, 0.58 mol) were heated at 100 °C under nitrogen for 3 h. The excess diamine was then eliminated under reduced pressure. The oily residue was dissolved in water. The pH was adjusted to pH 9 by means of 2 N sodium hydroxide solution. The aqueous solution was saturated with sodium chloride and extracted with methylene chloride. The organic phase was washed with brine and dried over magnesium sulfate. The solvent was removed, affording a yellow oil (11.5 g). The free base was crystallized by trituration with a mixture of 2-propanol and 2-propanol oxide. Mother liquors contain the symmetrically disubstituted amine and small amounts of free diamine. The product could also be purified by chromatography on silica gel. The symmetrically disubstituted compound was eluted with a mixture of ethyl acetate–methanol (75:25) and the expected product with pure methanol: mp 219–221 °C; yield 10.5 g (80%); IR (KBr) 3270, 2900, 2740, 2650, 1600, 1560 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.25 (d, J = 1.0 Hz, 3 H, HC=CH_3), 3.15 (t, J = 6.0 Hz, 2 H, H_2NCH_2), 3.75 (t, J = 6.0 Hz, 2 H, $HNCH_2$), 4.70 (br s, 2 H, exchangeable with D_2O, CH_2NH_2), 6.80 (br s, 1 H, exchangeable with D_2O, $NHCH_2$), 7.3–8.1 (m, 5 H, C_6H_5), 7.75 (q, J = 1.0, 1 H, HC=CH_3).

3-[(β-Aminopropyl)amino]-4-methyl-6-phenylpyridazine (27) and 3-[(4-aminobutyl)amino]-4-methyl-6-phenylpyridazine (29) were prepared in the same way, replacing ethylenediamine with propylenediamine and butylenediamine, respectively.

3-[(2-Aminopropyl)amino]-4-methyl-6-phenylpyridazine and 3-[[1-(Methyleneamino)ethyl]amino]-4-methyl-6-phenylpyridazine. The 75:25 mixture of these two isomers was obtained by heating 11 g (0.054 mol) of 3-chloro-4-methyl-6-phenylpyridazine and an excess of 40 g (0.54 mol) of 1,2-diaminopropane under argon at 100 °C for 3.5 h (it is also possible to add 1 equiv of ammonium chloride to shorten the reaction time to 2 h). The excess of diamine was then removed, affording a yellow oil. The mixture of isomers was isolated by chromatography on silica gel (eluent methanol–triethylamine, 95:5). Anal. C, H, N. ^{1}H NMR (DMSO-d_6): the 75:25 ratio between the two isomers was established by NMR. Main isomer: 1.08 (d, J = 6.0 Hz, 3 H, $CH(CH_3N)$, 2.22 (d, J = 1.0 Hz, 3 H, HC=CH_3), 2.45–2.85 (m, 4 H, $HNCH_2$ and protons exchangeable by D_2O, NH_2), 3.1–3.55 (m, 1 H, $HCNH_2$), 6.4 (t, J = 5.5 Hz, 1 H, $NHCH_2$), 7.3–8.2 (m, 6 H, HC=CH_3 and 6-phenyl). Minor isomer (25%): 1.26 (d, J = 6.0 Hz, 3 H, $HNCH(CH_3)CH_2$), 4.4–4.6 (m, 1 H, $HNCH(CH_3)CH_2$), 5.9 (d, J = 5.5 Hz, 1 H, $NHCH(CH_3)$).

Pharmacological Methods. Female albino mice (CD1 strain, Charles River France) weighing 18–22 g (reserpine and 5-HTP tests) or 25–30 g (turning) were used. The test compounds were dissolved or suspended in distilled water with gum tragacanth and administered intraperitoneally (ip) to groups of 7–20 mice per dose at multiple dose levels. The following tests were performed: evaluation of acute toxicity with graphic calculation of a 50% lethal dose (LD_{50}). Drugs or vehicle were injected to groups of 5–10 mice, which were then observed for 6–8 h. Lethality was recorded up to 72 h after drug administration.

Antagonism of reserpine-induced ptosis in mice was performed according to the method described by Gouret et al.[34] modified by Worms et al.[13] Drugs or vehicle were administered simultaneously with reserpine (2 mg/kg iv). One hour later, mice were observed individually for the occurrence or absence of palpebral ptosis, on an all-or-none basis (ptosis defined as closing of half, or more, of the palpebra). All control animals exhibited ptosis.

Potentiation of L-5-HTP-induced tremor in mice was performed according to the method described by Lessin.[35] Drugs or vehicle were injected 30 min before a threshold dose of L-5-HTP (200 mg/kg ip). Mice were isolated in small plexiglass cages (10 × 10 × 10 cm). Tremor was assessed on an all-or-none basis during the 20 min following L-5-HTP. Tremor occurred in 0–20% of the control animals.

Decrease of the spontaneous turning in mice bearing a unilateral 6-OHDA lesion of the striatum. For this latter test, the procedure described by Von Voigtlander and Moore[36] was slightly modified. The right nigrostriatal dopaminergic pathway was lesioned by stereotaxic injection of 10 μg (in 1 μL of saline containing 1 mg/mL of ascorbic acid) of 6-hydroxydopamine (6-OHDA) into the right striatum of anesthetized mice (pentobarbitone, 60 mg/kg ip). Seven days later, animals exhibited a spontaneous turning behavior, to the right (ipsilateral). At this time, the drugs or vehicle was injected ip, and the number of turns was counted 30 min later, during a period of 2 min. Control animals exhibited a rate of about 10 ipsilateral turns in 2 min. Percent antagonism of control values were calculated and data were expressed as minimal effective dose (MED) or the first dose that significantly decreased the spontaneous turning measured in controls ($p < 0.05$).

For the reserpine and 5-HTP models, effective 50% doses (ED 50) were calculated, with their 95% confidence limits, by log-probit analysis. The paired Student's t test was used to assess significance in the turning experiments.

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Registry No. **1**, 118269-66-2; **2**, 98-86-2; **4**, 13299-97-3; 4 (3-thienyl analogue), 118270-23-8; **5**, 28657-45-6; **6**, 13300-09-9; **7**, 28657-39-8; **12** (R_1 = Cl-*o*-Ph, R_2 = Ph), 118269-67-3; **14**, 94011-49-1; **15**, 34753-27-0; **16**, 34750-70-4; **17**, 118269-68-4; **17** (free base), 118270-50-1; **18**, 118269-69-5; **19**, 118269-70-8; **19** (free base), 118275-18-6; **20**, 87769-56-0; **21**, 94011-64-0; **22**, 94011-81-1; **22** (free base), 94011-82-2; **23**, 25905-57-1; **23** (free base), 25905-78-6; **24**, 118269-71-9; **24** (free base), 118270-24-9; **25**, 118269-72-0; **25** (free base), 118270-25-0; **26**, 118269-73-1; **26** (free base), 85714-56-3; **27**, 86662-99-9; **27** (free base), 86662-98-8; **28** (isomer 1), 86663-04-9; **28** (isomer 1, free base), 118270-26-1; **28** (isomer 2), 86663-03-8; **28** (isomer 2, free base), 118270-51-2; **29**, 86663-01-6; **29** (free base), 86663-00-5; **30**, 86672-44-8; **30** (free base), 118270-27-2; **31**, 118269-74-2; **31** (free base), 40064-50-4; **32**, 118269-75-3; **32** (free base), 40064-48-0; **33**, 118269-77-5; **33** (free base), 118269-76-4; **34**, 118269-78-6; **34** (free base), 118270-28-3; **35**, 118269-79-7; **35** (free base), 118270-29-4; **36**, 40064-51-5; **36** (free base), 40064-52-6; **37**, 40064-54-8; **37** (free base), 40064-55-9; **38**, 118269-80-0; **38** (free base), 118270-30-7; **39**, 118269-81-1; **39** (free base), 118270-31-8; **40**, 118269-82-2; **40** (free base), 118270-32-9; **41**, 118269-83-3; **41** (free base), 118270-33-0; **42**, 118269-84-4; **42** (free base), 118270-34-1; **43**, 118269-85-5; **43** (free base), 118270-35-2; **44**, 118269-86-6; **44** (free base), 118270-36-3; **45**, 118269-87-7; **45** (free base), 118270-37-4; **46**, 118269-88-8; **46** (free base), 118270-38-5; **47**, 118269-89-9; **47** (free base), 118270-39-6; **48**, 118269-90-2; **48** (free base), 118270-40-9; **49**, 118269-91-3; **49** (free base), 118270-41-0; **50**, 118269-92-4; **50** (free base), 118270-42-1; **51**, 118269-93-5; **51** (free base), 40064-53-7; **52**, 118269-94-6; **52** (free base), 118270-43-2; **53**, 118269-95-7; **53** (free base), 118270-44-3; **54**, 118269-96-8; **54** (free base), 118270-45-4; **55**, 118269-97-9; **55** (free base), 118270-46-5; **56**, 86663-17-4; **56** (free base), 82239-52-9; **57**, 86663-19-6; **57** (free base), 101969-83-9; **58**, 118269-98-0; **58**

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Fluoronaphthyridines and Quinolones as Antibacterial Agents. 1. Synthesis and Structure–Activity Relationships of New 1-Substituted Derivatives

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A series of novel 7-piperazinyl-1-substituted-6-fluoroquinolones and naphthyridines have been prepared and their antibacterial activities evaluated. These derivatives are characterized by having alkyl, alkenyl, arylalkyl, cycloalkyl, and cycloalkenyl groups at the 1-position. As a result of this study, derivatives **7** and **26**, which are substituted with *tert*-butyl groups at N-1, were found to possess excellent in vitro and in vivo potency, particularly against *Staphyloccus aureus*, comparable to that of norfloxacin (**1**) or ciprofloxacin (**10**). Structure–activity relationships of N-1 substituted alkyls and cycloalkyls are also discussed.

In the intense work on the class of quinolone antibacterials, basically two types of compounds can clearly be distinguished: the first group, typified by oxolinic acid and nalidixic acid, lack Gram-positive activity, while the second type, including norfloxacin[2] and enoxacin,[3] are compounds with relatively broad spectra. A number of new compounds, such as ciprofloxacin,[4] ofloxacin,[5] CI 934,[6] and difloxacin,[7] were prepared and tested, and many of them were found to be useful antibacterial agents and are in advanced development or already marketed.

These compounds share common structural features. Earlier[1] and more recent structure–activity relationship (SAR) studies[8,9] concluded that the optimal aliphatic group to attach to N-1 should be ethyl, vinyl, or a bioisostere of ethyl. Recently, several highly potent analogues have been developed that bring doubts and induce questions on the universality of this concept. Ciprofloxacin (*N*-cyclopropyl), ofloxacin (tricycle), and difloxacin (*N*-aryl) are examples of such compounds.

On the basis of former conclusions, systematic modification of the alkyl N-1 substituent received little attention. This laboratory explored and complemented data on the effect of the N-1 substituent on the antibacterial activity to update the SAR on quinolone antibacterials (see Figure 1a,b). In this paper, we report the synthesis and antibacterial activity of 7-(1-piperazinyl)-6-fluoro- and -6,8-difluoro-1-substituted-1,4-dihydro-4-oxoquinoline-3-carboxylic acids and 7-(1-piperazinyl)-6-fluoro-1-substituted-1,4-dihydro-4-oxo-1,8-naphthyridine-3-carboxylic acids (Tables I and II).

Chemistry

The general method used for the preparation of 4-oxoquinolines and naphthyridines is illustrated in Scheme I and was adapted from synthetic routes reported for analogues.[7,10,14–16] Reaction of ethyl 2-(2,4-dichloro-5-

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