Synthesis and Anticonvulsant Properties of New Benzylpyridazine Derivatives

Stéphane Moreau,† Pascal Coudert,† Catherine Rubat,‡ Daniel Gardette,§ Danielle Vallee-Goyet,§ Jacques Couquelet,*,† Pierre Bastide,‡ and Pierre Tronche†

Groupe de Recherches en Pharmacochimie, Laboratoire de Chimie Thérapeutique and Laboratoire de Pharmacologie et de Pharmacie Clinique, Faculté de Pharmacie, 28, Place Henri Dunant, 63001 Clermont-Ferrand Cedex France, and Laboratoire de Chimie des Substances Naturelles, URA C.N.R.S. 485, Université Blaise Pascal, 63177 Aubière Cedex France

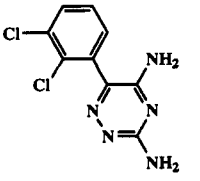
Received July 12, 1993•

Several 3-substituted pyridazines and a series of imidazo- and triazolopyridazines were synthesized and tested for anticonvulsant activity against maximal electroshock-induced seizures in mice. The most active derivatives, 3-ureidopyridazine **7** and triazolopyridazines **16**, **18**, **21**, and **25** with oral ED_{50}'s that ranged from 6.2 to 22.0 mg/kg, were more extensively investigated by evaluating their ability to prevent chemically induced seizures and were compared with phenytoin, phenobarbital, sodium valproate, carbamazepine, and diazepam. 3-amino-7-(2,6-dichlorobenzyl)-6-methyltriazolo-[4,3-*b*]pyridazine (**25**) was also protective in the pentylenetetrazole-induced seizures test (ED_{50} = 76 mg/kg per os) and blocked strychnine-induced tonic extensor seizures (ED_{50} = 34.5 mg/kg per os). Furthermore, derivative **25** showed anticonvulsant effects on bicuculline- and yohimbine-induced seizures tests in mice. All these results suggest that the pharmacological activity of **25** is partly due to modifications of glycinergic and GABAergic transmission. Moreover, molecular modeling studies based on the antiepileptic drug lamotrigine and the most stable conformer of **25** show structural similarities between these two molecules. This conformer also agrees with the electronic tolerances and volume of benzodiazepine pharmacophore models.

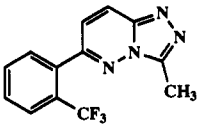
Although 70–80% of all epileptic patients are significantly benefited by currently available drugs, they often do so at the expense of adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity, gingival hyperplasia, hirsutism, and megaloblastic anemia.[1] Thus, a need currently exists for improved antiepileptic drugs with more selective anticonvulsant activity and lower toxicity. Therefore, the search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry.[2-9]

In a previous paper,[10] we reported the synthesis and anticonvulsant properties of new series of 5-substituted benzyl-2-pyridazinylacetamides and 2-pyridazinylacetohydrazides. In view to expand the scope of this investigation to other potential anticonvulsant pyridazine derivatives, we first examined the possibilities to introduce various substituents at the 3-position into the pyridazine ring. The most common structural elements of clinically active drugs against epilepsy appeared to be a nitrogen heteroatomic system bearing one or two phenyl rings and at least one carbonyl group. In order to agree with all these structural characteristics, we synthesized 3-ureido-, 3-semicarbazido-, and 3-hydrazidopyridazine derivatives.

Furthermore, description of the two potent anticonvulsant drugs lamotrigine[6] and 3-methyl-6-[2-(trifluoromethyl)phenyl]triazolo[4,3-*b*]pyridazine (CL 218.872)[3] prompted us to prepare chemically related benzyl imidazo- and benzyltriazolopyridazines and to evaluate their anticonvulsant properties.



Lamotrigine



CL 218.872

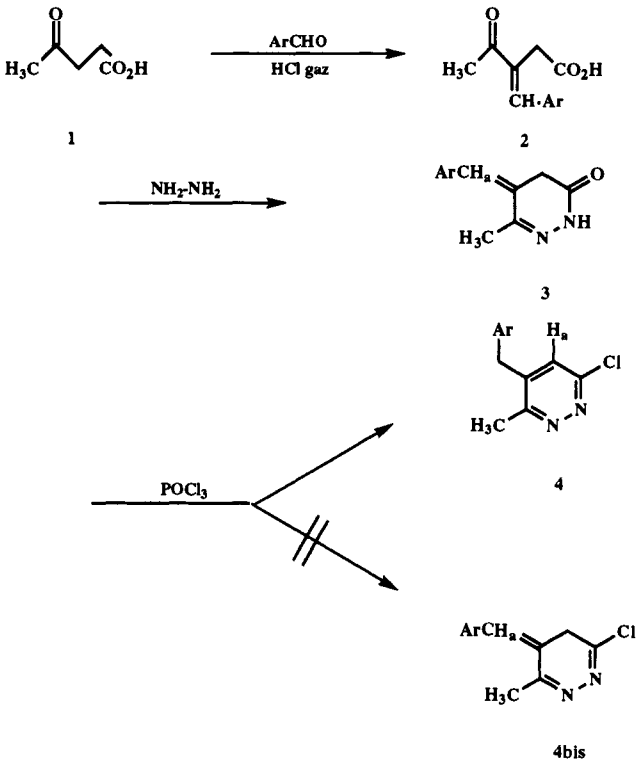
Chemistry

New series of benzylpyridazines (Table 1) were prepared from the key intermediate **4** using previously described synthetic methods (Schemes 1 and 2).[10-13] Reaction of levulinic acid (**1**) with aldehydes provided substituted acids **2**. Arylidenepyridazinones **3** were obtained in good yields by condensation of **2** with hydrazine hydrate. Treatment of **3** with phosphorus oxychloride led to *endo*-type compounds **4a–e**. The structure of **4a–e** was established by NMR and mass spectral analyses. The shift of proton Ha is higher in compounds **4** than in compounds **3** (7 ppm compared to 5.7–6.7 ppm). Irradiation at CHa causes significant increases of the area intensities of the methylenic protons of **4a** (Ar = C_6H_5) and **4a** (Ar = 2,6-diClC_6H_3). Positive NOE's are also observed between the methyl protons and the methylene group and between the methyl protons and the protons in the *ortho* position on the phenyl ring of **4a**. Another structural proof results from the observation of long-range coupling constants in the fully coupled ^{13}C NMR spectra between the carbon of the methylene group and both the proton Ha (^3J = 4.5 Hz in **4a**; ^3J = 4.0 Hz in **4e**) and the *o*-phenyl protons of **4a** (^3J = 4.5 Hz). Moreover, the mass spectra of **4a,e** differ from those of the corresponding arylidenepyridazinones **3a,e**, mainly by a greater abundance of the tropylium ion. This abundance is respectively 66% and 40% for **4a** and **4e** and 21% for both **3a** and **3e**.

† Laboratoire de Chimie Thérapeutique.
‡ Laboratoire de Pharmacologie et de Pharmacie Clinique.
§ Laboratoire de Chimie des Substances Naturelles.
• Abstract published in *Advance ACS Abstracts*, June 1, 1994.

0022-2623/94/1837-2153$04.50/0

Scheme 1



Synthesis of the new compound **7** was carried out by reacting 3-aminopyridazine **5** (Ar – C_6H_5) with potassium cyanate in boiling acetic acid. Reaction of **5** with 2-bromoacetophenone resulted in formation of imidazopyridazine **8**.

Preparation of 3-(phenylsemicarbazido)pyridazines **9** and **10** was achieved by action of phenyl isocyanate with 3-hydrazinopyridazines **6** in chloroform at room temperature. Semicarbazidopyridazine **11** (R = -$N(C_6H_5)_2$) was synthesized by direct acylation of **6** with diphenylcarbamoyl chloride in the presence of triethylamine. Acylation of **6** with isobutyryl chloride gave (isobutyrohydrazido)pyridazines **12** and **13** (R = -$CH(CH_3)_2$). Similarly, treatment of **6** with benzoyl chloride furnished (benzoylhydrazino)pyridazine **14** (R = C_6H_5). Cyclization of **14** in phosphorus oxychloride at 120–130 °C afforded phenyltriazolopyridazine **15**. Heating of **6** in formic acid provided triazolopyridazines **16–18**. Compound **6** also reacted with triethyl orthoacetate, leading to methyltriazolopyridazines **19** and **20**. Aminotriazolopyridazines **21–25** were synthesized conveniently in a single step by condensing **6** with cyanogen bromide followed by alkalinization with concentrated aqueous ammonia. Acylation of **21** (Ar = C_6H_5) with benzoyl chloride, carried out in anhydrous chloroform, resulted in (benzoylamino)triazolopyridazine **26**.

For compounds **21** (Ar = C_6H_5) and **25** (Ar = 2,6-diClC_6H_3), NOE effects and the fully coupled ^{13}C NMR spectra were similar to those observed for **4a,e**.

Results and Discussion

The initial evaluation of the potential anticonvulsant pyridazine derivatives was performed using the maximal electroshock seizure (MES) test which is claimed to detect compounds of value in treating grand mal and partial seizures.[14] Except for the semicarbazido- and the hydrazidopyridazine compounds **9–14**, all other derivatives produced significant anticonvulsant activity with oral ED_{50}'s that ranged from 6 to 108 mg/kg (Table 1). Compound **7** with a urea moiety at the 3-position in the pyridazine ring was very effective with an ED_{50} value of 10.2 mg/kg. The most active bicyclic compounds were 3-unsubstituted triazolopyridazines **16–18** (6.0 mg/kg ≤ ED_{50} ≤ 9.5 mg/kg). The presence of a methyl substituent (**19, 20**) or an amino group (**21–25**) in the triazole ring did not considerably affect anticonvulsant properties with respect to the MES test. Moreover, introduction of a phenyl group at the 3-position of the triazole ring (**15**) resulted in a decrease of activity, suggesting that the limits of steric bulk had been reached. Likewise, replacement of an amino group (**21**) by a benzoylamino moiety (**26**) reduced MES activity. In contrast, the addition of one or two chloro substituents in an *ortho* position on the benzene ring (**17, 18, 22, 25**) appeared to increase the anticonvulsant effects, compared to unsubstituted derivatives **16** and **21**. At last, the same activity was found as well for imidazopyridazine **8** as for triazolopyridazine **15**, showing that the presence of an imidazole or triazole nucleus in the bicyclic structure as well as a phenyl ring in the 2- or 3-position had no perceptible effect on anticonvulsant activity.

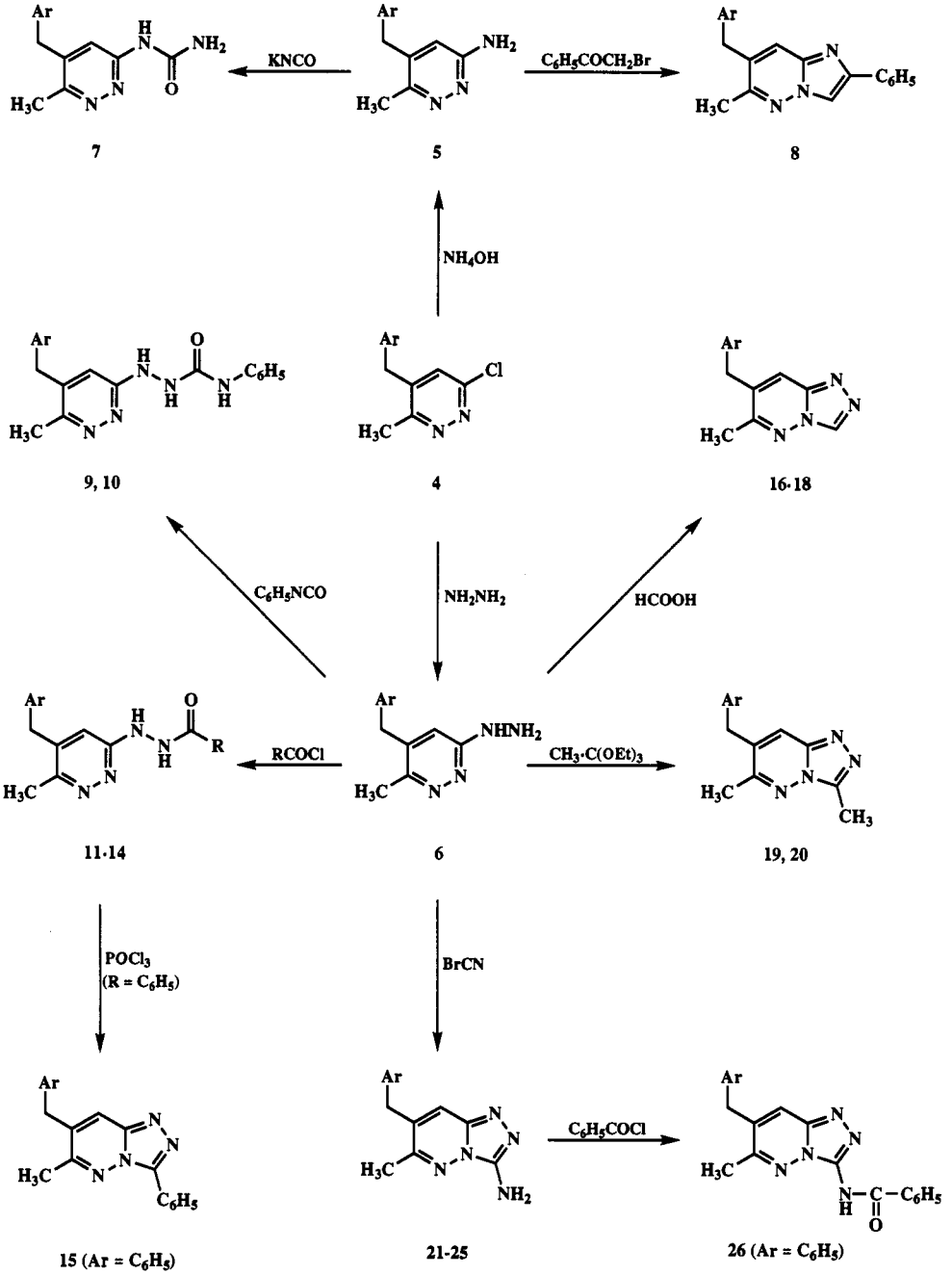
Spontaneous motor activity was measured as a parameter of the sedative action of compounds in the central nervous system (Table 1). The most active drugs induced either a very slight increase or a decrease in spontaneous motor activity at 100 mg/kg. Only compound **19** was significantly active, producing a 47% increase in motor activity. These results are particularly interesting since many anticonvulsant drugs (i.e., phenytoin, phenobarbital, and diazepam) affect motor movements at therapeutic doses.

As a result of these first tests, compounds **7, 16, 18, 21,** and **25** were selected for further investigations. Quantification of their neurotoxicity in mice was performed by determining the median toxic dose (TD_{50}) in the rotarod test. These data are shown in Table 2. Data for several currently marketed anticonvulsants are also included for comparison. The smaller TD_{50} values for **7, 6,** and **18** resulted in protective indices of about 14–18 which were nevertheless double that of phenytoin, phenobarbital, and carbamazepine tested under similar conditions. Due to their larger protective indices, 3-aminotriazolopyridazines **21** and **25** appeared to offer greater therapeutic potential as anticonvulsants than the three other derivatives.

Among the possible mechanisms which are known to inhibit seizure activity are enhancement of GABA transmission and antagonism of glutamate transmission.[15] Therefore, for a third time, activity against chemically induced seizures was investigated for the five selected compounds (Table 3). Strychnine, bicuculline, yohimbine, and *N*-methyl-D,L-aspartate (NMDLA) correspond to inhibition by glycine, GABA inhibitory function, and amino acid-mediated excitation, respectively.[15,16]

In the pentylenetetrazole test, derivatives **18** and **25** protected 50% of the mice against seizures at 70 and 76 mg/kg, respectively. Therefore, the presence of two chlorine atoms on the benzyl moiety appeared particularly efficient for anticonvulsant properties. It is also remarkable to note that these two compounds showed a higher anticonvulsant activity in this test than the clinically useful antiepileptic drugs phenytoin and sodium valproate. The result of the pentylenetetrazole test suggests an antiabsence effect for compounds **18** and **25**,[17] which is also a hallmark of benzodiazepine agonists.[16]

Scheme 2



Against strychnine-induced seizures, the five pyridazine derivatives were active with oral ED_{50}'s that ranged from 28 to 85 mg/kg. The ED_{50}'s of carbamazepine and diazepam were 8.5 and 1.1 mg/kg, respectively, while other reference substances were inactive.

The five selected pyridazines were effective in the bicuculline test. Interestingly, (2,6-dichlorobenzyl)triazolopyridazine **18** was less active than the parent unsubstituted compound **16**, whereas 3-aminotriazolopyridazines **21** and **25** were equipotent with ED_{50}'s of 94 and 93 mg/kg, respectively. Thus, the influence of phenyl ring substitution by two chlorine atoms was not straightforward for anticonvulsant potency in this test. Compared with compound **7**, it also protected mice against clonic seizures, showing ED_{50} = 111 mg/kg. Whereas phenytoin, sodium valproate, and carbamazepine failed to protect animals from seizures, phenobarbital and diazepam provided better protection than the pyridazines did.

In the yohimbine-induced clonic seizures test, only compounds **18** and **25** were active with ED_{50} values equal to 150 and 78 mg/kg, respectively. The anticonvulsant activities of the five reference substances were comparable to that found in the bicuculline test.

As the yohimbine-induced seizures seemed to be mediated through the impairment of GABAergic transmission and also through an endogenous enhancement of excitatory amino acid transmission,[18] we examined possible antagonism of NMDLA-induced convulsions by the pyridazine derivatives. It appeared that all compounds failed to significantly protect animals from NMDLA-induced seizures. Most of the common antiepileptic drugs were also ineffective in this test, except phenobarbital which showed an ED_{50} value of 30 mg/kg, orally. By the same way, diazepam was weakly active and protected only 30% of the mice from seizures at the neurotoxic dose of 30 mg/kg.

Table 1. Physical Constants, Locomotor Activity, and Anticonvulsant Activity of Pyridazine Derivatives in the Maximal Electroshock Seizure Test (MES)

compd	Ar	R	mp, °C	yield, %	formula	MES ED_{50}, mg/kg per os	locomotor activity at 100 mg/kg per os, (−) = decrease, (+) = increase
7	C_6H_5		240	50	$C_{13}H_{14}N_4O$	10.2 (7.5–13.9)[c]	−5 ± 3 (NS)
8	C_6H_5		152	74	$C_{20}H_{17}N_3 \cdot 0.5H_2O$	105 (53–210)[c]	+12 ± 1 (NS)
9	C_6H_5		210	60	$C_{19}H_{19}N_5O$	0[d]	+9 ± 4 (NS)
10	2-ClC_6H_4		200	81	$C_{19}H_{18}N_5ClO$	33[b,d]	−3 ± 2 (NS)
11	C_6H_5	$N(C_6H_5)_2$	231	15	$C_{25}H_{23}N_5O$	15 (NS)[d]	+9 ± 5 (NS)
12	C_6H_5	$CH(CH_3)_2$	175	77	$C_{16}H_{20}N_4O \cdot HCl$	0[d]	−67 ± 4[a]
13	2-ClC_6H_4	$CH(CH_3)_2$	190	82	$C_{16}H_{19}N_4ClO \cdot HCl$	17 (NS)[d]	−57 ± 3[a]
14	C_6H_5	C_6H_5	194	89	$C_{19}H_{18}N_4O \cdot HCl$	17 (NS)[d]	−62 ± 3[a]
15	C_6H_5		188	78	$C_{19}H_{16}N_4 \cdot 1.5H_2O$	108 (70–168)[c]	+2 ± 1 (NS)
16	C_6H_5		150	87	$C_{13}H_{12}N_4 \cdot 1.5H_2O$	9.5 (6.9–13.1)[c]	+8 ± 3 (NS)
17	2-ClC_6H_4		109	89	$C_{13}H_{11}N_4Cl$	6.0 (3.0–12.0)[c]	+5 ± 3 (NS)
18	2,6-diClC_6H_3		186	67	$C_{13}H_{10}N_4Cl_2$	6.2 (3.3–11.6)[c]	+13 ± 5 (NS)
19	C_6H_5		196	85	$C_{14}H_{14}N_4$	26.5 (23.6–29.8)[c]	+47 ± 4[b]
20	2-ClC_6H_4		164	72	$C_{14}H_{13}N_4Cl$	30.5 (24.2–38.5)[c]	−1 ± 1 (NS)
21	C_6H_5		238	63	$C_{13}H_{13}N_5$	22.0 (16.8–28.8)[c]	+11 ± 4 (NS)
22	2-ClC_6H_4		251	64	$C_{13}H_{12}N_5Cl$	17.4 (12.4–24.5)[c]	−7 ± 4 (NS)
23	4-$CH_3C_6H_4$		216	67	$C_{14}H_{15}N_5 \cdot H_2O$	22.5 (15.5–32.7)[c]	−8 ± 5 (NS)
24	4-FC_6H_4		212	85	$C_{13}H_{12}N_5F \cdot 2H_2O$	43.0 (32.3–57.3)[c]	−15 ± 6 (NS)
25	2,6-diClC_6H_3		228	51	$C_{13}H_{11}N_5Cl_2 \cdot 2H_2O$	9.2 (6.3–13.1)[c]	+16 ± 4 (NS)
26	C_6H_5		132	25	$C_{20}H_{17}N_5O \cdot 0.5H_2O$	35.5 (30.4–41.5)[c]	+7 ± 3 (NS)
phenytoin						4.0 (3.0–5.3)[c]	−16 ± 5[b,e]
phenobarbital						4.6 (3.0–7.0)[c]	+33 ± 4[a,e]
sodium valproate						112.0 (88.2–142.2)[c]	−5 ± 2 (NS)
carbamazepine						5.5 (4.6–6.5)[c]	+7 ± 3 (NS)[f]
diazepam						4.0 (3.4–4.8)[c]	−42 ± 6[a,e]

[a] The level of significance was $p < 0.001$. [b] The level of significance was $p < 0.05$. NS = not significant. [c] 95% confidence intervals. [d] Protection percentage at 100 mg/kg po. [e] Tested at 5 mg/kg po. [f] Tested at 10 mg/kg po.

Table 2. Rotarod Test and Protective Index for Compounds 7, 16, 18, 21, and 25

compd	rotarod TD_{50},[a] mg/kg per os	PI[b]
7	140.0 (111.1–176.4)	13.7
16	170.0 (145.9–198.1)	17.9
18	88.0 (46.7–165.8)	14.2
21	>800	36.4
25	410.0 (332.3–505.9)	44.5
phenytoin	30.5 (22.7–40.9)	7.6
phenobarbital	33.0 (29.3–37.2)	7.2
sodium valproate	208.0 (173.8–248.9)	1.9
carbamazepine	40.0 (38.3–41.8)	7.3
diazepam	2.8 (1.8–4.2)	0.7

[a] Numbers in parentheses are 95% confidence intervals. [b] PI (protective index) = TD_{50}/ED_{50}. PI values are for MES test.

All these results indicate that the pharmacological activity of the most active compound, **25**, was widely due to modifications of glycinergic and GABAergic transmission.

Furthermore, computer-assisted molecular modeling was used to determine structural analogies between lamotrigine and **25** by way of the molecular modeling software SYBYL 6.3,[19] as described in the Experimental Section. First, the input geometries were generated and initially minimized with the Tripos force field.[19] A conformational analysis was carried out for derivative **25**. Rotation around the C_2–C_3 (θ_1) and C_3–C_4 (θ_2) bonds from 0° to 180° gave a three-dimensional (E,θ_1,θ_2) graph. Its two-dimensional projection on the E,θ_1 plane gave a curve with two minima of close energies with an energy barrier of 12 kJ mol^{-1}. Further refinement using AM1 calculation shows that the conformer with the phenyl group away from the methyl group (Figure 2) is more stable by more than 8 kJ mol^{-1}. Superimposition of this conformer and lamotrigine, also optimized by the AM1 method, was performed with the FIT procedure within SYBYL.

The structural features taken into account for the matching processes were one of the chlorine atoms and the center of the triazole ring for **25**. For lamotrigine, the *m*-chlorine atom and the center of the triazine ring were chosen in the modeling studies; the fitting experiments showed a good superimposition (Figure 2; RMS = 0.184).

As a further attempt to demonstrate structural similarities between **25** and lamotrigine, we considered their atomic charges which were computed by the program MOPAC. Considering both superimposed chlorine atoms of the two molecules, we found that the atomic charges were very much alike, with −0.001 e and 0.008 e values for **25** and lamotrigine, respectively (Figures 3 and 4).

In the case of lamotrigine, the guanidinic nitrogen atoms of the triazole ring carried charges of −0.159 e and −0.285 e. A charge of −0.345 e was found for the primary amine of the guanidinic moiety. The corresponding values in **25** were found to be respectively −0.110 e, −0.105 e, and −0.272 e, showing a comparable electronic distribution in the two structures. The charge of the carbon atom bearing the second amino group of lamotrigine was 0.188 e versus −0.042 e for the corresponding nitrogen atom of **25**.

Considering isopotential maps of both molecules generated by SYBYL from MOPAC charges, important differences appeared (Figures 5 and 6). Negative and positive areas of **25** and lamotrigine did not effectively occupy the same regions. This last result could partly explain why lamotrigine prevented NMLDA-induced seizures[6] whereas triazolopyridazine **25** was almost inactive. However, the interaction sites of both molecules were located in the same three-dimensional region, making possible a similar interaction with the benzodiazepine receptor complex.

Therefore, we compared **25** with the essential structural features of the pharmacophoric models of Brandau and Tebib (Figures 7 and 8).[20,21] Although different pharmacophoric models of the central benzodiazepine receptor were described,[20–23] they all presented the following common features:

(1) a planar aromatic region (PAR),

Table 3. Anticonvulsant Activity (ED_{50}, mg/kg) of Compounds **7**, **16**, **18**, **21**, and **25** in the Chemically Induced Seizures Tests

compd	pentylenetetrazole[f]	strychnine	bicuculline	yohimbine	NMDLA
7	20 (NS)[b]	28.0 (22.0–35.6)[c]	111.0 (95.9–128.4)[c]	10 (NS)[d]	12.5 (NS)[d]
16	20 (NS)[b]	85.0 (74.6–96.8)[c]	63.0 (39.7–99.9)[c]	0[d]	25 (NS)[d]
18	70.0 (44.8–109.5)[c]	39.5 (29.0–53.8)[c]	125.0 (67.3–232.3)[c]	150.0 (103.4–217.7)[c]	12.5 (NS)[d]
21	20 (NS)[b]	35.0 (28.2–43.5)[c]	94.0 (79.2–111.6)[c]	20 (NS)[d]	14 (NS)[d]
25	76.0 (64.4–89.7)[c]	34.5 (26.0–45.7)[c]	93.0 (55.2–156.7)[c]	76.0 (66.5–86.8)[c]	14 (NS)[d]
phenytoin	0[b]	0[b]	0[b]	10 (NS)[d]	0 (NS)[d]
phenobarbital	15.0 (13.4–16.8)[c]	0[b]	20.0 (14.7–27.2)[c]	17.0 (12.1–23.9)[c]	30.0 (12.8–70.5)[c]
sodium valproate	20 (NS)[b]	0[b]	14 (NS)[b]	10 (NS)[d]	0 (NS)[d]
carbamazepine	17.5 (12.1–25.2)[c]	8.5 (7.6–9.6)[c]	0[b]	10 (NS)[b]	20 (NS)[d]
diazepam	0.5 (0.3–0.8)[c]	1.1 (0.6–1.8)[c]	0.39 (0.24–0.64)[c]	2.3 (1.4–3.7)[c]	30[a,e]

[a] The level of significance was $p < 0.05$. NS = not significant. [b] Percentage of protection at 100 mg/kg per os. [c] 95% confidence intervals. [d] Percentage of protection at 150 mg/kg per os. [e] Percentage of protection at 30 mg/kg per os. [f] Administered at the dose of 106.25 mg/kg sc.

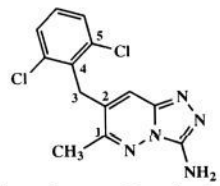


Figure 1. Dihedral angles used in the initial conformational analysis of **25** by the multitorsion grid search method of SYBYL.

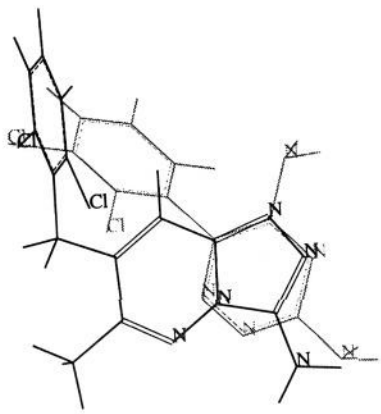


Figure 2. Superimposition of compound **25** (bold solid) and lamotrigine (solid).

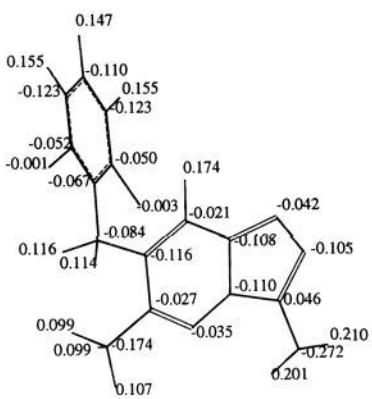


Figure 3. Atomic charges of compound **25** computed by the program MOPAC.

(2) two electron-rich zones, E_1 and E_2, placed respectively at about 5.0 and 4.5 Å from the reference centroid A in the PAR zone, and

(3) a lipophilic pocket which was an out-of-plane region (OPR) strongly associated with agonist properties.

As *stereochemical requirements for classical benzodi*azepines were similar to those of the triazolopyridazine series,[21] the amino substituent in **25** had to be placed in the E_1 zone and the nitrogen atoms N_1 and N_2 in the E_2 region. The 2,6-dichlorophenyl ring was out of the triazolopyridazine plane and could cover the OPR zone.

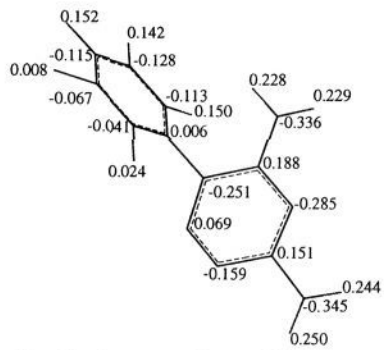


Figure 4. Atomic charges of lamotrigine computed by the program MOPAC.

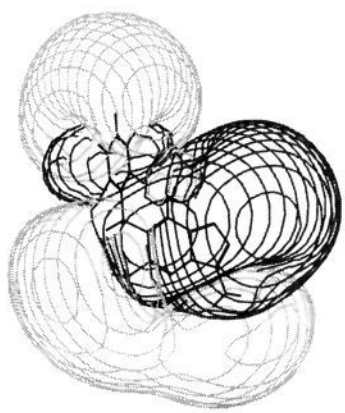


Figure 5. Stereoview of electrostatic isopotential surfaces (E) of compound **25**. $E \leq -1$ kcal/mol; bold solid. $E \geq 1$ kcal/mol; solid.

In conclusion, we have investigated new various series of pyridazine derivatives, particularly triazolopyridazines which were potent anticonvulsant compounds. Molecular modeling studies revealed evident structural similarities between **25** and lamotrigine, suggesting possible interactions of **25** with the benzodiazepine receptor complex. This observation confirms the interest of amino heterocyclic compounds as anticonvulsant drugs, while the currently accepted opinion was that the most obvious structural feature necessary for anticonvulsant activity is a carbonyl moiety,[24] found in the common antiepileptic drugs.

Experimental Section

Chemical Methods. Melting points were taken on a calibrated Kofler apparatus and are uncorrected. The infrared spectra were recorded on a Beckman 4240 spectrophotometer. The proton nuclear magnetic resonance spectra were performed on a Varian EM 360 A spectrometer using the δ scale with reference to

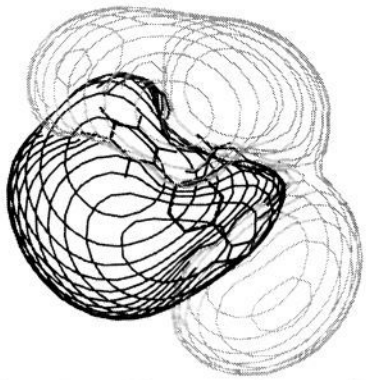


Figure 6. Stereoview of electrostatic isopotential surfaces (E) of lamotrigine. $E \leq -1$ kcal/mol; bold solid. $E \geq 1$ kcal/mol; solid.

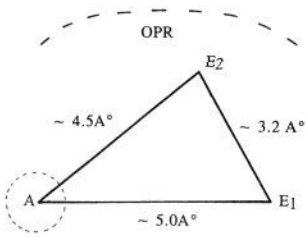


Figure 7. Pharmacophoric model of the central benzodiazepine receptor.

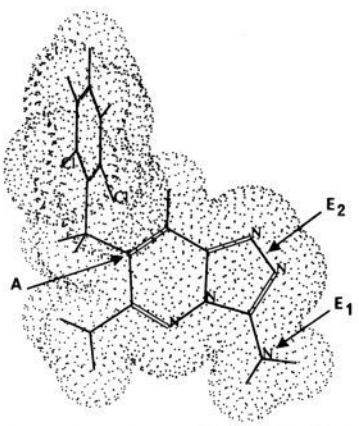


Figure 8. Perspective view and van der Waals volume of compound **25**. A–E_1 = 5.2 Å, A–E_2 = 4.1 Å, and E_1–E_2 = 3.0 Å.

tetramethylsilane for DMSO-d_6 solution. NOE difference spectra and ^{13}C and ^1H–^{13}C spectra were recorded on a Brücker 300 MSL. Mass spectra were obtained on a HP 5989 A mass spectrometer. The ionizing energy was 70 eV, and samples were introduced by direct insertion. TLC analysis of derivatives was carried out on silica gel plates (60 F_{254}; E. Merck, Darmstadt, Germany) with the following solvent system: ethyl acetate/methanol 95:5, v/v. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on TLC. Elemental analyses were performed by the Service Central d'Analyses du C.N.R.S. de Vernaison-France, and all analytical data were within ±0.4% of the theoretical values.

3-Ureido-5-benzyl-6-methylpyridazine (7). A solution of potassium cyanate (0.41 g, 5 mmol) in water (3 mL) was added to 0.99 g (5 mmol) of 3-amino-5-benzyl-6-methylpyridazine (**5**) in glacial acetic acid (15 mL). The mixture was heated for 24 h in a 100 °C bath, cooled, poured on ice, and, then, basified to pH 7.5 with ammonium hydroxide (20% aqueous solution). The precipitate which separated was filtered off, washed with ethyl ether, and recrystallized from ethanol. IR (KBr): 3460–3100, 1640 cm^{-1}. ^1H NMR: δ 2.40 (s, 3H, CH_3), 3.70 (br s, 2H, NH_2), 4.10 (s, 2H, CH_2), 6.35 (br s, 1H, NH), 7.35–7.60 (m, 6H, C_6H_5 + CH=). Anal. C, H, N.

2-Phenyl-6-methyl-7-benzylimidazo[3,2-*b*]pyridazine (8). A mixture of 3-amino-5-benzyl-6-methylpyridazine (**5**) (1.19 g, 6 mmol) and 2-bromoacetophenone (1.19 g, 6 mmol) in absolute ethanol (90 mL) was refluxed for 30 h. After cooling, the solid obtained was filtered and dissolved in an ethanolic solution (40 mL) of sodium (0.18 g, 8 mmol). The mixture was refluxed for 1 h. After cooling, the precipitate formed was filtered off, washed with water, and recrystallized from ethanol. IR (KBr): 3120, 1600 cm^{-1}. ^1H NMR: δ 2.45 (s, 3H, CH_3), 3.35 (br s, 1H, $0.5H_2O$), 4.10 (s, 2H, CH_2), 7.30–8.10 (m, 11H, $2C_6H_5$ + CH=), 8.70 (s, 1H, NCH=). Anal. (0.5 hydrate) C, H, N.

Typical Procedure. 3-(4-Phenylsemicarbazido)-5-benzyl-6-methylpyridazine (9). A solution of 3-hydrazino-5-benzyl-6-methylpyridazine (**6**) (2.67 g, 12.5 mmol) and phenyl isocyanate (1.36 g, 12.5 mmol) in chloroform (50 mL) was stirred at room temperature for 24 h. The obtained precipitate was filtered off, washed with ethyl ether, and recrystallized from a mixture of ethanol/water 30:70. IR (KBr): 3260, 1710 cm^{-1}. ^1H NMR: δ 2.50 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 6.80 (s, 1H, CH=), 7.00–7.80 (m, 5H, C_6H_5), 8.30 (br s, 1H, NH-C_6H_5), 8.60 (br s, 1H, NH-NHCO), 8.90 (br s, 1H, NH-NHCO). Anal. C, H, N.

3-(4,4-Diphenylsemicarbazido)-5-benzyl-6-methylpyridazine (11). A solution of 3-hydrazino-5-benzyl-6-methylpyridazine (**6**) (2.14 g, 10 mmol), diphenylcarbamoyl chloride (2.31 g, 10 mmol), and triethylamine (1.01 g, 10 mmol) in chloroform (100 mL) was heated at reflux for 24 h. The chloroform was evaporated from the reaction mixture and the residue dissolved in absolute ethanol (15 mL). After addition of ethyl ether (200–300 mL) to the ethanolic solution, the solid which separated was filtered off and recrystallized from ethanol. IR (KBr): 3200–3000, 1700 cm^{-1}. ^1H NMR: δ 2.45 (s, 3H, CH_3), 4.00 (s, 2H, CH_2), 6.45 (s, 1H, CH), 7.30–7.40 (s, 15H, $3C_6H_5$), 8.20 (br s, 1H, NHCO), 8.40 (br s, 1H, NH). Anal. C, H, N.

Typical Procedure. 3-(3-Isobutyrohydrazido)-5-benzyl-6-methylpyridazine (12). A solution of 3-hydrazino-5-benzyl-6-methylpyridazine (**6**) (2.67 g, 12.5 mmol) and isobutyryl chloride (1.33 g, 12.5 mmol) was stirred at room temperature for 24 h. After evaporation, the residue was triturated with ethyl ether until crystallization. The resulting solid was collected by filtration, washed with acetone, and recrystallized from ethanol. IR (KBr): 3500, 2900, 1670 cm^{-1}. ^1H NMR: δ 1.20 (d, 6H, $2CH_3$), 2.60 (m, 1H, CH), 2.70 (s, 3H, CH_3), 4.30 (s, 2H, CH_2), 7.20 (s, 1H, CH=), 7.50 (m, 5H, C_6H_5), 10.7 (m, 3H, NHCO + NH_2^+). Anal. (hydrochloride) C, H, N, Cl.

3-(2-Benzoylhydrazino)-5-benzyl-6-methylpyridazine (14). A solution of 3-hydrazino-5-benzyl-6-methylpyridazine (**6**) (2.67 g, 12.5 mmol) and benzoyl chloride (1.78 g, 12.5 mmol) was stirred at room temperature for 24 h. The obtained precipitate was collected by filtration, washed with anhydrous ethyl ether, and recrystallized from ethanol. IR (KBr): 3200–3000, 2400, 1670 cm^{-1}. ^1H NMR: δ 2.60 (s, 3H, CH_3), 4.25 (s, 2H, CH_2), 7.40–7.70 (m, 11H, $2C_6H_5$ + CH=), 8.10 (br s, 2H, NH_2^+), 11.40 (br s, 1H, NHCO). Anal. (hydrochloride) C, H, N, Cl.

3-Phenyl-6-methyl-7-benzyltriazolo[4,3-*b*]pyridazine (15). A mixture of compound **14** (1.4 g, 4 mmol) and phosphorus oxychloride (10 mL) was heated at 120 °C for 3 h. The solution was poured into ice water, and the precipitate which separated was filtered off. Then, the product was washed with aqueous sodium carbonate (0.5 N) and recrystallized from ethanol. IR (KBr): 3400, 1600 cm^{-1}. ^1H NMR: δ 2.55 (s, 3H, CH_3), 3.70 (br s, 3H, $1.5H_2O$), 4.25 (s, 2H, CH_2), 7.40–8.50 (s, 11H, $2C_6H_5$ + CH=). Anal. (1.5 hydrate) C, H, N.

Typical Procedure. 6-methyl-7-benzyltriazolo[4,3-*b*]pyridazine (16). A solution of 3-hydrazino-5-benzyl-6-methylpyridazine (**6**) (2.14 g, 10 mmol) in formic acid (30 mL) was heated under reflux for 2 h 30 min. After evaporation, the residue was tritured with ethyl ether until crystallization. The product was filtered and recrystallized from a mixture of ethanol/water 50:50. IR (KBr): 3400, 1630 cm^{-1}. ^1H NMR: δ 2.45 (s, 3H, CH_3), 3.40 (br s, 3H, $1.5H_2O$), 4.15 (s, 2H, CH_2), 7.35 (m, 5H, C_6H_5), 7.90 (s, 1H, CH=), 9.5 (s, 1H, NCH=). Anal. (1.5 hydrate) C, H, N.

Typical Procedure. 3,6-Dimethyl-7-benzyltriazolo[4,3-*b*]pyridazine (19). A mixture of 3-hydrazino-5-benzyl-6-methylpyridazine (**6**) (2.14 g, 10 mmol) and an excess of triethyl orthoacetate (20 mL) was heated under reflux for 2 h. After cooling, the resultant solid was filtered, washed with ethyl ether,

and recrystallized from a mixture of ethanol/water 50:50. IR (KBr): 1630 cm^{-1}. ^1H NMR: δ 2.45 (s, 3H, CH_3), 2.70 (s, 3H, N=C(CH_3)N), 4.15 (s, 2H, CH_2), 7.35 (m, 5H, C_6H_5), 7.90 (s, 1H, CH=). Anal. C, H, N.

Typical Procedure. 3-Amino-6-methyl-7-benzyltriazolo-[4,3-*b*]pyridazine (21). A solution of 3-hydrazino-5-benzyl-6-methylpyridazine (6) (3.21 g, 15 mmol) and cyanogen bromide (1.80 g, 17 mmol) in ethanol (50 mL) was stirred for 5 h at room temperature. After cooling, the pH of the solution was adjusted to 10 with concentrated aqueous ammonium hydroxide. A precipitate slowly appeared, which was filtered, washed with ethyl ether, and recrystallized from ethanol. IR (KBr): 3320, 1640 cm^{-1}. ^1H NMR: δ 2.45 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 6.50 (br s, 2H, NH_2), 7.40–7.70 (m, 6H, C_6H_5 + CH=). Irradiation of the methyl protons caused increases of areas intensities of CH_2 and *ortho* aromatic protons. Positive NOE was observed for methylene protons when =CH was irradiated; similar enhancements existed for methyl, *ortho* aromatic protons, and the =CH proton when methylenic protons were irradiated. Long-range coupling constants (^3J) were observed between ^{13}C of the methylene group and both *ortho* aromatic and =CH protons. Anal. C, H, N.

3-(Benzoylamino)-6-methyl-7-benzyltriazolo[4,3-*b*]pyridazine (26). A solution of 21 (1.2 g, 5 mmol) and benzoyl chloride (0.71 g, 5 mmol) in anhydrous chloroform (20 mL) was stirred for 24 h at room temperature. After evaporation, the residue was triturated with ethyl ether until crystallization. Then, the product was collected by filtration and recrystallized from ethanol. IR (KBr): 3300, 3200, 1690 cm^{-1}. ^1H NMR: δ 2.45 (s, 3H, CH_3), 4.20 (s, 2H, CH_2), 6.30 (br s, 1H, $0.5H_2O$), 7.35–8.10 (m, 11H, $2C_6H_5$ + CH=), 11.20 (br s, 1H, NH). Anal. (0.5 hydrate) C, H, N.

Pharmacological Methods. All compounds were administered orally in a 0.5% hydroxypropyl methyl cellulose suspension to Iffa Credo OF_1 male mice weighing 20 g. Groups of 10 mice at each dose were used.

Sedative Activity. This activity was evaluated by a determination of spontaneous motor activity. The test was performed by the method of Boissier and Simon[25] in photoelectric activity cages (Apelex). Test drugs were administered 30 min before evaluation of spontaneous motor activity, and the number of passages was scored during 10 min.

Neurotoxicity. The rotarod test[26] was used to evaluate central nervous system toxicity. Test drugs were administered 30 min before assay. Neurologic toxicity was defined as the failure of the dosed animal to remain on a 3-cm diameter wood rod rotating at 6 rpm for 3 min.

Anticonvulsant Activity. Compounds were tested for their ability to protect mice against electrically and chemically induced seizures.

Effect on Maximal Electroshock Seizures.[6] Test drugs and vehicles were administered orally 30 min before subjecting the animals to maximal electroshock through corneal electrodes. Protection against seizures was defined as the abolition of the hind limb tonic extensor component of seizures.

Effect on Seizures Induced by Pentylenetetrazole.[6,27] Test compounds were administered orally 30 min before evaluation for their ability to prevent the tonic extensor component induced by 106.25 mg/kg sc of pentylenetetrazole. Anticonvulsant activity was judged when the component was blocked.

Effect on Seizures Induced by Strychnine.[28] The ability of the test compounds to provide a protection against seizures was measured 30 min after administration. Protection was defined as the abolition of the hand leg tonic extensor component of the seizure induced by a 0.96 mg/kg sc injection of strychnine.

Effect on Seizures Induced by Bicuculline.[29] The antagonism of bicuculline-induced seizures was evaluated according to a procedure similar to that described by Heyer. Compounds were administered 30 min before the test. Bicuculline was administered intravenously at a dose of 0.81 mg/kg. Protection against clonic seizures was recorded.

Effect on Seizures Induced by Yohimbine.[18] For the antagonism of yohimbine seizure studies, compounds were administered 30 min before the test. Yohimbine was administered subcutaneously at a dose of 45 mg/kg. Animals that did not exhibit at least one clonic seizure within 60 min were considered protected.

Effect on Seizures Induced by NMDLA.[30] Test compounds were given 30 min prior to injection of 345 mg/kg sc of *N*-methyl-D,L-aspartate. Protection against full generalized tonic seizures was recorded.

Data Analysis. Statistical analysis of the results was performed using the method of Schwartz.[31] The data on the spontaneous motor activity were analyzed by using the Student's *t*-test. All values were expressed as mean ± SD. The data of electrically and chemically induced seizures as well as the data of rotarod test were analyzed by means of the chi-square test with Yates correction. The ED_{50} and TD_{50} values were determined using the method of Litchfield and Wilcoxon.[32]

Molecular Modeling Studies. The topographical and electrostatic characterization of the studied molecules was performed using the SYBYL 6.3 software package on a Silicon Graphics Personal IRIS 4D35TG workstation. Structures were built within SYBYL and minimized by MAXIMIN2 with the Tripos force field, in vacuo conditions, to provide reasonable standard geometries. Molecules were deemed to be minimized when there was a minimum energy charge of less than 0.021 kJ mol^{-1} for one iteration. The conjugate gradient method was used for minimization. All AM1 calculations involved the singlet state. Molecules were deemed to be minimized when the gradient fell to less than 0.021 kJ mol^{-1}. The conformational spaces of lamotrigine and 25 were explored using the SYBYL search facility. Torsion angles were defined around the single bonds C_2–C_3 and C_4–C_5 of 25, and a grid search was performed allowing these bonds to rotate with a 180° revolution by 15° increments. The lowest-energy conformers thus obtained were submitted to AM1 calculations (MOPAC version 5.0)[33] to optimize their geometry and determine atomic charge distributions.

A structural fitting between lamotrigine and 25 was done by using the FIT option of SYBYL. The molecular electrostatic potential surfaces were calculated in SYBYL and partitioned into two intervals as follows: −1 kcal mol^{-1} and down, and 1 kcal mol^{-1} and up.

References

(1) Davies-Jones, G. A. B. Anticonvulsants. In *Meyler's side effects of drugs*, 11th ed.; Dukes, M. N. G., Ed.; Elsevier Science Publishers B.V.: New York, 1988; pp 120–136.

(2) Mergen, F.; Lambert, D. M.; Goncalves Saraiva, J. C.; Poupaert, J. H.; Dumont, P. Antiepileptic activity of 1,3-dihexadecanoylamino-2-valpropyl-propan-2-ol, a prodrug of valproic acid endowed with a tropism for the central nervous system. *J. Pharm. Pharmacol.* **1991**, *43*, 815–816.

(3) Keir, D. I. B.; Ong, J. Gaba agonists and antagonists. *Med. Res. Rev.* **1992**, *12*, 593–636.

(4) Palmer, G. C.; Harris, E. W.; Ray, R.; Stagnitto, M. L.; Schmiesing, R. J. Classification of compounds for prevention of NMDLA-induced seizures/mortality, or maximal electroshock and pentylenetetrazol seizures in mice and antagonism of MK801 binding in vitro. *Arch. Int. Pharmacodyn. Ther.* **1992**, *317*, 16–34.

(5) Thurkauf, A.; Newman, A. H.; Calderon, S. N.; De Costa, B. R.; Rice, K. C.; Rogawski, M. A.; Yamaguchi, S. I. Synthesis and anticonvulsant activity of 4-amino-(*N*-1-aryl-cycloalkyl)-benzamides. *Med. Chem. Res.* **1992**, *2*, 63–69.

(6) Brodie, J. M. Lamotrigine. *Lancet* **1992**, *339*, 1397–1400.

(7) Kehne, J. H.; Kane, J. M.; Miller, F. P.; Ketteler, H. J.; Braun, D. L.; Senyah, Y.; Chaney, S. F.; Abdallah, A.; Dudley, M. W.; Ogden, A. M.; Palfreyman, M. G. MDL 27,531 selectively reverses strychnine-induced seizures in mice. *Br. J. Pharmacol.* **1992**, *106*, 910–916.

(8) Ornstein, P. L.; Schoepp, D. D.; Arnold, M. B.; Augenstein, N. K.; Lodge, D.; Millar, J. D.; Chambers, J.; Campbell, J.; Paschal, J. W.; Zimmerman, D. M.; Leander, J. D. 6-Substituted decahydroisoquinoline-3-carboxylic acids as potent and selective conformationally constrained NMDA receptor antagonists. *J. Med. Chem.* **1992**, *35*, 3547–3560.

(9) Edafiogho, I. O.; Hinko, C. N.; Chang, H.; Moore, J. A.; Mulzac, D.; Nicholson, J. M.; Scott, K. R. Synthesis and anticonvulsant activity of enaminones. *J. Med. Chem.* **1992**, *35*, 2798–2805.

(10) Rubat, C.; Coudert, P.; Refouvelet, B.; Tronche, P.; Bastide, P.; Bastide, J. Anticonvulsant activity of 3-oxo-5-substituted benzylidene-6-methyl-(4*H*)-2-pyridazinylacetamides and 2-pyridazinylacetylhydrazides. *Chem. Pharm. Bull.* **1990**, *38*, 3009–3013.

(11) Couquelet, J. M.; Taoufik, J.; Couquelet, J. D.; Tronche, P.; Pharm Huu, C.; Pharm Huu, A.; Navarro-Delmasure, C.; Lasserre, B. Nouveaux dérivés des dihydro-4,5 (3*H*) acétyl-4 furannones-2: étude

chimique et pharmacologique. (New 4,5-dihydro-4-acetyl-2(3*H*)-furanone derivatives: chemical and pharmacological studies.) *Eur. J. Med. Chem.* **1983**, *18*, 301–305.
(12) Taoufik, J.; Couquelet, J. D.; Couquelet, J. M.; Carpy, A. Stereospecific Synthesis of New 5-Substituted 6-Methyl-4,5-dihydro-2*H*-pyridazin-3-ones. X-Ray Assignment Study. *J. Heterocycl. Chem.* **1984**, *21*, 305–310.
(13) Taoufik, J.; Couquelet, J. M.; Couquelet, J. D.; Tronche, P. Arylidene-5 methyl-6 4*H* pyridazines substituées en 3 et systèmes à noyaux condensés. (3-Substituted-5-arylidene-6-4*H*-pyridazines.) *J. Heterocycl. Chem.* **1985**, *22*, 1615–1619.
(14) Krall, R. L.; Peury, J. K.; White, B. G.; Kupferberg, M. J.; Swinyard, E. A. Antiepileptic drug development: II. Anticonvulsant drug screening. *Epilepsia* **1978**, *19*, 405–428.
(15) Rall, T. W.; Schleifer, L. S. Drugs effective in the therapy of the epilepsies. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Macmillan Publishing Co.: New York, 1990; pp 436–462.
(16) Rall, T. W. Hypnotics and sedatives; ethanol. In *The Pharmacological Basis of Therapeutics*, 8th ed.; Gilman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Macmillan Publishing Co.: New York, 1990; pp 345–382.
(17) Bleck, T. P.; Klawans, H. L. Convulsive disorders: mechanisms of epilepsy and anticonvulsant action. *Clin. Neuropharmacol.* **1990**, *13*, 121–128.
(18) Dunn, R. W.; Corbett, R. Yohimbine-induced seizures involve NMDA and GABA ergic transmission. *Neuropharmacology* **1992**, *31*, 389–395.
(19) Tripos Associates, Inc., 1699 S. Harley Rd., Suite 303, St. Louis, MO 63144.
(20) Brandau, B.; Bourguignon, J. J.; Wermuth, C. G. Ligands of the central benzodiazepine receptors: computer-aided characterisation of their specific interactions. In *QSAR: Rational approaches to the design of bioactive compounds*; Silipo, C., Vittoria, A., Eds.; Elsevier Science Publishers B. V.: Amsterdam, 1991; pp 253–256.
(21) Tebib, S.; Bourguignon, J. J.; Wermuth C. G. The active analog approach applied to the pharmacophore identification of benzodiazepine receptor ligands. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 153–170.
(22) Hollinshead, S. P.; Trudell, M. L.; Skolnick, P.; Cook, J. M. Structural requirements for agonist action at the benzodiazepine receptor: studies with analogues of 6-(benzyloxy)-4-(methoxymethyl)-β-carboline-3-carboxylic acid ethyl ether. *J. Med. Chem.* **1990**, *33*, 1062–1069.
(23) Vilar, H. O.; Uyeno, E. T.; Toll, L.; Polgar, W.; Davies, M. F.; Loew, G. H. Molecular determinants of benzodiazepine receptor affinities and anticonvulsant activities. *Mol. Pharmacol.* **1989**, *36*, 589–600.
(24) Kane, J. M.; Baron, B. M.; Dudley, M. W.; Sorensen, S. M.; Staeger, M. A.; Miller, F. P. 2,4-Dihydro-3*H*-1,2,4-triazol-3-ones as anticonvulsant agents. *J. Med. Chem.* **1990**, *33*, 2772–2777.
(25) Boissier, J. R.; Simon, P. Action de la caféine sur la motilité spontanée de la souris. (Caffeine effects on spontaneous motor activity in mice.) *Arch. Int. Pharmacodyn. Ther.* **1965**, *158*, 212–221.
(26) Kinnard, W. J.; Carr, C. J. A preliminary procedure for the evaluation of central nervous system depressants. *J. Pharmacol. Exp. Ther.* **1957**, *121*, 354–361.
(27) Swinyard, E. A.; Brown, W. C.; Goodman, L. S. Comparative assays of antiepileptic drugs in mice and rats. *J. Pharmacol. Exp. Ther.* **1952**, *106*, 319–330.
(28) Gouret, C.; Linee, P. Etude de trois tests rapides de sélection d'une activité anticonvulsivante chez la souris. (Three rapid tests for selection of drugs with anticonvulsant activity in mice.) *J. Pharmacol. (Paris)* **1972**, *3*, 57–72.
(29) Heyer, E. J.; Nowak, L. M.; MacDonald, R. L. Bicuculline: a convulsant with synaptic and non synaptic actions. *Neurology* **1981**, *31*, 1381–1390.
(30) Czuczwar, S. J.; Frey, H. H.; Löscher, W. Antagonism of N-Methyl-D,L-Aspartic acid-induced convulsions by antiepileptic drugs and other agents. *Eur. J. Pharmacol.* **1985**, *108*, 273–280.
(31) Schwartz, D. *Méthodes statistiques à l'usage des médecins et des biologistes*; Editions médicales Flammarion: Paris 1984; pp 263–307.
(32) Litchfield, J. T.; Wilcoxon, E. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99–113.
(33) Merz, K. M.; Besler, B. H. MOPAC version 5.0 ESC, QCPE n°589, 1990, with AM1 force field parameters.