Anticonvulsant Activity of 3-Oxo-5-substituted Benzylidene-6-methyl-(4*H*)-2-pyridazinylacetamides and 2-Pyridazinylacetylhydrazides

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A series of 3-oxo-5-substituted-benzylidene-6-methyl-(4*H*)-2-pyridazinylacetamides and 2-pyridazinylacetylhydrazides were synthesized and evaluated for anticonvulsant activity against electrically and chemically induced seizures. In the maximal electroshock-induced seizures test, most of the derivatives showed an anticonvulsant effect better than that of sodium valproate, a commonly used anticonvulsant drug. At 100 mg/kg orally, compounds 5a and 5b respectively protected 50 and 60% of the mice against pentylentetrazole-induced seizures. In addition, these two derivatives showed significant anticonvulsant properties at doses that did not produce ataxia or sedation. The title compounds were also tested for their ability to antagonize convulsions induced by bicuculline and strychnine. Their effect on tremors induced by oxotremorine in mice was also evaluated.

Keywords substituted benzylidene pyridazinone; pyridazine derivative; 2-pyridazinylacetamide; 2-pyridazinylacetylhydrazide; anticonvulsant activity; sedative activity; neurotoxicity

The search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry.[1-5] Although several drugs are used in the treatment of epilepsy, many patients fail to experience satisfactory seizure control with them, or they do so at the expense of significant side effects.[6,7] Due to the need for improved antiepileptic drugs, many compounds having diverse chemical structures have been evaluated. These compounds often have structural features quite different from the more popular antiepileptic drugs *viz.* carbamazepine, phenytoïn, phenobarbital and sodium valproate.[8] A closer look reveals the presence of an amide moiety, cyclic or not, in most anticonvulsants. The pyridazinone ring system agrees with this salient feature and many papers have reported anticonvulsant properties of pyridazine derivatives.[9-11]

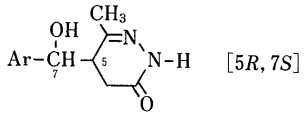
These last considerations prompted us to synthesize new compounds containing a 5-substituted benzylidene pyridazinone moiety and to evaluate their anticonvulsant properties. Some of them were found to be effective in anticonvulsant activity tests and devoid of sedative effects and neurotoxicity.

Chemistry

The 5-arylidene-6-methyl-(4*H*)-pyridazin-3-ones substituted by an acetamido or an acetylhydrazido group were synthesized by the methods shown in Chart 1.

In previous papers[12-14] we have reported the preparation of 5-substituted benzylidene-6-methyl-(4*H*)-pyridazin-3-ones. This synthesis was achieved in three steps starting from α-angelicalactone which was condensed with aromatic aldehydes using boron trifluoride as a catalyst. In order to improve preparation of 5-arylidene pyridazinones, we have developed a new synthetic route starting from levulinic acid **1**. The latter was condensed with aromatic aldehydes in the presence of dry hydrogen chloride and without a solvent. The resulting substituted benzylidene levulinic acids **2** were refluxed in ethanol with hydrazine hydrate to afford the expected pyridazinones **3**.

Compounds **3** are not mixtures of the two isomers (*E*) and (*Z*) as shown by thin layer chromatography (TLC), and are identical with those obtained by the method starting from α-angelicalactone. An X-ray crystal study published in a previous paper[13] supported the assignment of configuration erythro [5*R*,7*S*] for their hydroxylated precursors prepared from α-angelicalactone:



[5*R*, 7*S*]

So, dehydratation of these hydroxypyridazinones involving a transelimination should very likely lead to arylidene pyridazinones (*E*). On the other hand, an *endo* type of compound such as structure **3**bis was excluded because the

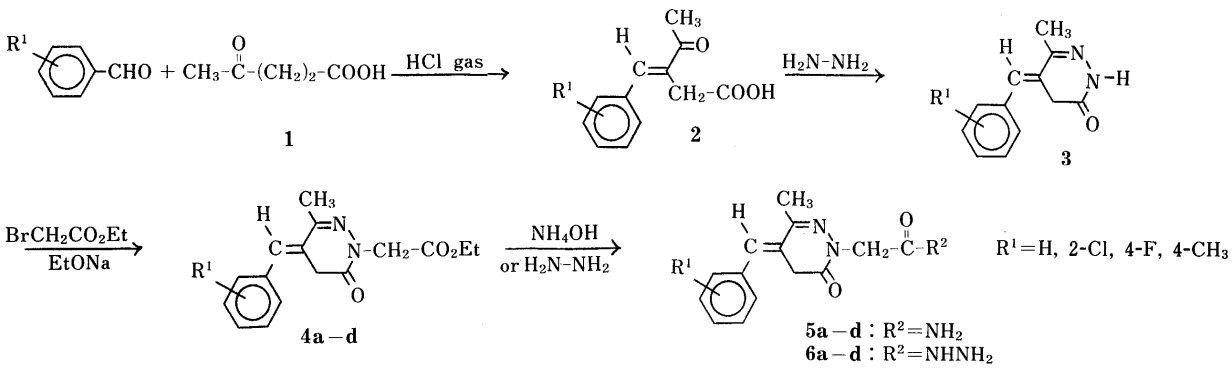


Chart 1

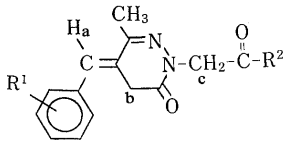
chemical shifts observed for the ethylenic proton H_a were not compatible with the aromatic form **3bis**.
Chemical shifts of proton Ha ranged from 5.7 to 6.7 ppm,[15] whereas in aromatic structures comparable to **3bis**, literature data indicate chemical shifts superior to 7 ppm for H_a.[16] In addition, variations of chemical shifts for H_a explain themselves in the case of structure **3** by the direct influence of substituents of the phenyl nucleus. In order to bring about a definitive proof of the configuration of pyridazinones **3**, the nuclear Overhauser effect (NOE) difference spectroscopy was measured in case of pyridazinone **3a** ($R^1=H$).[14] Irradiation of the methyl protons did not significantly change the intensity of other signals. On the other hand, irradiation of the methylene group concommitantly caused an increase of the intensity of the ethylenic proton and the two protons in the *ortho* position on the phenyl nucleus. In addition, when the ethylenic proton was irradiated, NOE was observed at the signals of the methylene group and of the two *ortho* aromatic protons.

3 3 bis

TABLE I. Physical Constants of 5-Substituted Benzylidene-6-methyl-(4*H*)-pyridazinone Derivatives

Compd. No.	R^1	R^2	Yield (%)	mp (°C)	Formula	Analysis (%) Calcd C	H	F/Cl	N	Found C	H	F/Cl	N
4a	H	OC_2H_5	98	90	$C_{16}H_{18}N_2O_3$	67.13	6.29		9.79	67.42	6.30		9.63
4b	2-Cl	OC_2H_5	75	77	$C_{16}H_{17}ClN_2O_3$	59.91	5.30	11.07	8.74	59.55	5.20	11.17	8.56
4c	4-F	OC_2H_5	96	98	$C_{16}H_{17}FN_2O_3$	63.16	5.59	6.25	9.21	63.20	5.52	6.11	9.40
4d	4-CH_3	OC_2H_5	82	130	$C_{17}H_{20}N_2O_3$	68.00	6.67		9.33	68.11	6.69		9.40
5a	H	NH_2	55	160	$C_{14}H_{15}N_3O_2\cdot 1/2H_2O$	63.16	6.01		15.79	62.99	5.96		15.84
5b	2-Cl	NH_2	66	118	$C_{14}H_{14}ClN_3O_2\cdot 1/2H_2O$	55.91	4.99	11.81	13.98	56.08	5.03	11.96	13.84
5c	4-F	NH_2	53	200	$C_{14}H_{14}FN_3O_2\cdot 1/2H_2O$	59.15	5.28	6.69	14.79	59.08	5.15	6.82	14.91
5d	4-CH_3	NH_2	76	202	$C_{15}H_{17}N_3O_2$	68.70	6.48		16.03	68.65	6.31		15.97
6a	H	$NHNH_2$	67	167	$C_{14}H_{16}N_4O_2\cdot 1/2H_2O$	59.92	5.84		19.92	59.92	5.80		19.69
6b	2-Cl	$NHNH_2$	65	205	$C_{14}H_{15}ClN_4O_2$	54.81	4.90	11.58	18.27	54.83	4.85	11.45	18.11
6c	4-F	$NHNH_2$	59	170	$C_{14}H_{15}FN_4O_2\cdot 1/2H_2O$	56.19	5.35	6.35	18.73	56.06	5.27	6.55	18.69
6d	4-CH_3	$NHNH_2$	66	190	$C_{15}H_{18}N_4O_2$	62.94	6.29		19.58	63.09	6.30		19.37

TABLE II. Spectral Data for 5-Substituted Benzylidene-6-methyl-(4*H*)-pyridazinone Derivatives



Compd. No.	R^1	R^2	IR ν_{max}^{KBr} cm^{-1}	^{1}H-NMR Chemical shift (δ) (in DMSO-d_6)
4a	H	O-$\underset{d}{CH_2}$-$\underset{e}{CH_3}$	1750, 1660	1.25 (t, 3H, e), 2.20 (s, 3H, CH_3), 3.90 (s, 2H, b), 4.10 (q, 2H, d), 4.75 (s, 2H, c), 6.50 (s, 1H, Ha), 7.30 (m, 5H, Ar)
4b	2-Cl	O-$\underset{d}{CH_2}$-$\underset{e}{CH_3}$	1750, 1670	1.25 (t, 3H, e), 2.25 (s, 3H, CH_3), 4.05 (s, 2H, b), 4.20 (q, 2H, d), 4.80 (s, 2H, c),6.15 (s, 1H, Ha), 7.45 (m, 4H, Ar)
4c	4-F	O-$\underset{d}{CH_2}$-$\underset{e}{CH_3}$	1745, 1660	1.30 (t, 3H, e), 2.30 (s, 3H, CH_3), 4.10 (s, 2H, b), 4.30 (q, 2H, d), 4.80 (s, 2H, c), 6.60 (s, 1H, Ha), 7.25 (m, 4H, Ar)
4d	4-$\underset{f}{CH_3}$	O-$\underset{d}{CH_2}$-$\underset{e}{CH_3}$	1745, 1660	1.15 (t, 3H, e), 2.15 (s, 3H, f), 2.30 (s, 3H, CH_3), 3.80 (s, 2H, b), 4.15 (q, 2H, d), 4.70 (s, 2H, c), 6.45 (s, 1H, Ha), 7.10 (m, 4H, Ar)
5a	H	NH_2	3360, 3150, 1670, 1640	2.25 (s, 3H, CH_3), 3.50 (br s, 1H, 1/2H_2O), 3.90 (s, 2H, b), 4.55 (s, 2H, c), 6.45 (s, 1H, Ha), 7.40 (m, 5H, Ar), 7.50 (br s, 2H, NH_2)
5b	2-Cl	NH_2	3380, 3140, 1670, 1650	2.30 (s, 3H, CH_3), 3.70 (br s, 1H, 1/2H_2O), 4.10 (s, 2H, b), 4.60 (s, 2H, c), 6.15 (s, 1H, Ha), 7.00 (br s, 2H, NH_2), 7.50 (m, 4H, Ar)
5c	4-F	NH_2	3340, 3140, 1670, 1640	2.30 (s, 3H, CH_3), 3.50 (br s, 1H, 1/2H_2O), 3.90 (s, 2H, b), 4.70 (s, 2H, c), 6.55 (s, 1H, Ha), 7.10 (br s, 2H, NH_2), 7.50 (m, 4H, Ar)
5d	4-$\underset{f}{CH_3}$	NH_2	3340, 1685, 1655	2.20 (s, 3H, f), 2.30 (s, 3H, CH_3), 3.85 (s, 2H, b), 4.60 (s, 2H, c), 6.50 (s, 1H, Ha), 7.20 (m, 4H, Ar), 7.50 (br s, 2H, NH_2)
6a	H	$NHNH_2$	3300, 1660, 1650	2.25 (s, 3H, CH_3), 3.90 (s, 2H, b), 4.10 (br s, 3H, NH_2 and 1/2H_2O), 4.60 (s, 2H, c), 6.50 (s, 1H, Ha), 7.35 (m, 5H, Ar), 9.25 (br s, 1H, NH)
6b	2-Cl	$NHNH_2$	3320, 1660, 1655	2.30 (s, 3H, CH_3), 4.10 (s, 2H, b), 4.25 (br s, 2H, NH_2), 4.70 (s, 2H, c), 6.20 (s, 1H, Ha), 7.50 (m, 4H, Ar), 9.30 (br s, 1H, NH)
6c	4-F	$NHNH_2$	3320, 1660, 1655	2.30 (s, 3H, CH_3), 4.00 (s, 2H, b), 4.25 (br s, 3H, NH_2 and 1/2H_2O), 4.70 (s, 2H, c), 6.55 (s, 1H, Ha), 7.35 (m, 4H, Ar), 9.30 (br s, 1H, NH)
6d	4-$\underset{f}{CH_3}$	$NHNH_2$	3300, 1660, 1650	2.15 (s, 3H, f), 2.25 (s, 3H, CH_3), 3.80 (s, 2H, b), 4.20 (br s, 2H, NH_2), 4.50 (s, 2H, c), 6.10 (s, 1H, Ha), 7.10 (m, 4H, Ar), 9.20 (br s, 1H, NH)

All these observations supported the configuration (*E*) for pyridazinone **3a**.

Preparation of pyridazinones **4a—d** was achieved by a nucleophilic substitution of the hydrogen atom attached in the 2-position of the pyridazine ring by ethyl bromacetate. Reaction of *N*-substituted pyridazinones **4a—d** with ammonia or hydrazine hydrate respectively provided compounds **5a—d** and **6a—d**. The derivatives **4**, **5** and **6** are listed in Table I. Their structure was established from analytical and spectral data (Tables I and II).

Biological Results and Discussion

Behavioral effects and oral acute toxicity were first investigated in mice. In addition, neurotoxicity was evaluated by measuring the ability of the tested animal to remain on a rotating rod. The titled compounds were evaluated for anticonvulsant activity in mice against maximal electroshock-induced seizures (MES). Sedative effects were estimated by a study of spontaneous motor activity. In a second test period, most active compounds were screened for their ability to antagonize pentylenetetrazole-, bicuculline-, strychnine-induced seizures and also an oxotremorine-induced tremor. Pharmacological data are summarized in Tables III and IV.

TABLE III. Spontaneous Motor Activity, Anticonvulsant Activity against Maximal Electroshock Seizures in Mice of Compounds **4a—d**, **5a—d**, **6a—d** and Their Relative Lipophilicity (R_M)

Compd. No.	Effect on motor activity (%) (−) decrease (+) increase	Electroshock	R_M
	100 (mg/kg)	ED_{50} (mg/kg)	
4a	2±1 (NS)	>100	0.07
4b	−6±2 (NS)	>100	0.21
4c	−8±3 (NS)	>100	0.10
4d	11±5 (NS)	Inactive	0.27
5a	16±5[b]	13.5 (7.4−24.5)[c]	−0.10
5b	−8±4 (NS)	10.0 (8.1−12.3)[c]	0.05
5c	14±3[b]	61.0 (46.2−80.8)[c]	−0.07
5d	13±6 (NS)	33.5 (29.6−37.8)[c]	0.14
6a	18±6[b]	55.0 (40.7−74.3)[c]	0.09
6b	−17±5[b]	35.5 (28.1−44.7)[c]	0.24
6c	−11±5 (NS)	>100	0.12
6d	73±4[a]	42.5 (34.5−52.2)[c]	0.27
Phenytoin	−16±5[b] at 5 mg/kg	4.0 (3.0− 5.3)[c]	Not tested
Phenobarbital	30±4[a] at 10 mg/kg	4.6 (3.0− 7.0)[c]	Not tested
Valproate	20±6[b] at 100 mg/kg	>100	Not tested
Carbamazepine	−7±3 (NS) at 10 mg/kg	5.5 (4.6− 6.5)[c]	Not tested

The level of significance was *a*) $p<0.001$, *b*) $p<0.05$ or (NS) not significant *c*) 95% confidence intervals.

No significant behavioral effects were observed even at doses up to 800 mg/kg orally except with compound **6d**. In the case of the derivative **6d**, from 100 mg/kg the animals presented an important excitation that disappeared after 24 h. With other compounds tested at the dose of 800 mg/kg, all animals were still alive after an observation period of one week. No neurologic toxicity was observed at 100 mg/kg in the rotarod test with all derivatives tested.

Spontaneous motor activity was measured as a parameter of the sedative action of compounds in the central nervous system area (Table III). Most of the tested drugs provoked either a very slight decrease or an increase in spontaneous motor activity at 100 mg/kg. Only compound **6d** was significantly active, producing a 73% increase in motor activity.

Most of the *N*-substituted pyridazinones possessed significant anti-convulsant activity blocking MES. The most potent molecules occurred in the acetamido and acetylhydrazido derivatives. Compounds **5a—d** with an amide chain in the 2-position of the pyridazine ring were more potent than their hydrazido homologues **6a—d**. Compounds **5a** and **5b** with a benzylidene and a 2-chlorobenzylidene moiety on the pyridazine ring were the most active derivatives with an ED_{50} value of 13.5 and 10 mg/kg orally respectively. The activity was less potent than that of phenyltoïn, phenobarbital and carbamazepine but it was several times higher than that of valproate.

The variant degree of anticonvulsant activity against MES exhibited by pyridazinones cannot be directly related to their lipophilicity (Table III). It is evident that the nature of the chain attached in the 2-position of the pyridazine ring remarkably influenced the activity. But compounds **5a** and **5c**, which possess a comparable relative lipophilicity and the same amide side chain, showed significantly different anticonvulsant activity, suggesting the importance of other parameters such as substitution on the phenyl ring.

In order to explore more extensively the anticonvulsant activity of the most active pyridazinones, protection against chemically induced-seizures was also investigated (Table

TABLE IV. Protection against Seizures Induced by Pentylenetetrazole, Bicuculline, Strychnine and against Tremor Induced by Oxotremorine

Compd. No.	Pentylenetetrazole 100 (mg/kg) (%)	Bicuculline 100 (mg/kg) (%)			Strychnine 100 (mg/kg) (%)	Oxotremorine 100 (mg/kg) (%)
		Clonic seiz.	Tonic seiz.	Lethality		
5a	50[b]	50[b]	100[a]	75[a]	40[c]	45[c]
5b	60[b]	27 (NS)	91[a]	73[a]	10 (NS)	25 (NS)
5c	30 (NS)	42[c]	75[a]	75[a]	Inactive	10 (NS)
5d	20 (NS)	8 (NS)	75[a]	67[a]	30 (NS)	28 (NS)
6a	20 (NS)		Not tested		Inactive	33 (NS)
6b	10 (NS)		Not tested		Inactive	22 (NS)
6c	Inactive		Not tested		Inactive	Inactive
6d	10 (NS)		Not tested		Inactive	11 (NS)
Phenytoin	Inactive	Inactive	100[a]	50[b]	Inactive	Inactive
Phenobarbital	15.0 (13.4−16.8)[d]	50[b,e]	90[a,e]	80[a,e]	Inactive	31.0 (25.8−37.2)[d]
Valproate	20 (NS)	50[b,f]	80[a,f]	80[a,f]	Inactive	Inactive
Carbamazepine	17.5 (12.1−25.2)[d]	Inactive	100[a]	100[a]	70[a]	Inactive

The level of significance was: *a*) $p<0.001$, *b*) $p<0.01$, *c*) $p<0.05$ or (NS) not significant. *d*) ED_{50} with 95% confidence intervals. *e*) Tested at 20 mg/kg. *f*) Tested at 200 mg/kg.

IV). In the pentylenetetrazole test, compounds **5a** and **5b** protected respectively 50% and 60% of the mice against seizures at 100 mg/kg. It is remarkable to note that these two derivatives showed a higher anticonvulsant activity in this test than the clinically useful anticonvulsants sodium valproate and phenytoin. Parasubstitution of the phenyl nucleus with an electron withdrawing substituent or an electron-donating substituent resulted in a loss of the activity (compounds **5c**, **5d**). Pyridazinyl acetylhydrazides **6a—d** did not exhibit significant anticonvulsant activity.

Derivative **5a** was the most active compound of the series of pyridazinylacetamides in the bicuculline test. At 100 mg/kg orally, it protected against the clonic seizure phase in 50% of animals and completely antagonized the tonic hind limb extension, suggesting a possible GABAergic mechanism. It provided better protection to animals than did phenytoin and valproate and was approximately equipotent at a 100 mg/kg orally dose level to a 20 mg/kg orally dose of phenobarbital. At the same dose of 100 mg/kg orally, carbamazepine did not protect mice from clonic seizures caused by a threshold dose of subcutaneous bicuculline.

Against strychnine-induced seizures, only compound **5a** was effective at 100 mg/kg. All the other derivatives were inactive or did not show significant anticonvulsant effects. Carbamazepine protected 70% of the mice in this test, while other reference substances were inactive.

Furthermore, the effect on tremors induced by oxotremorine was investigated. Pyridazinone **5a** exhibited significant activity at 100 mg/kg: suppression of the tremor was observed with 45% of the animals. This compound was inactive on tear secretion and the salivation of animals and did not modify hypothermia induced by oxotremorine. Analogues of **5a** with substituents attached to the phenyl ring were clearly less active in antagonizing tremors induced by oxotremorine. Pyridazinones **6a—d** were found to have low activity in this test. In comparison, the median effective dose for phenobarbital is 31 mg/kg orally, whereas phenytoin, sodium valproate and carbamazepine are inactive.

In conclusion, compound **5a** and a number of analogues represent a new class of potential orally active anticonvulsant agents with valuable activity against MES in mice. Derivative **5a** is also effective against seizures induced by chemical agents and it possesses significant anticonvulsant properties at doses that do not produce ataxia or sedation. This new class of orally active anticonvulsant agents provides a novel lead for the development of compounds that may be useful in the treatment of seizure disorders for which drugs such as phenytoin, phenobarbital, sodium valproate or carbamazepine are presently indicated.

Experimental

Melting points were determined on a Kofler apparatus and were uncorrected. The infrared (IR) spectra were recorded on a Beckman 4240 spectrophotometer. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian EM 360 A spectrometer. Resonance positions are given δ on the scale (parts per million) relative to internal tetramethylsilane. The NMR signals were designated as follows: s, singlet; br s, broad singlet; t, triplet; q, quartet; m, multiplet. NOE difference spectra were taken on a Bruker 300 MSL and measured with 4 s irradiation.

4-Oxo-3-substituted Benzylidene-pentanoic Acids (2) An ice-cooled mixture of aromatic aldehyde (0.2 mol) and levulinic acid (0.3 mol) was saturated with dry hydrogen chloride. Then, the mixture was stirred for 24 h at room temperature. The precipitate which was formed was filtered off and washed with ethyl ether. The crude acids **2** were used without further purification.

5-Substituted Benzylidene-6-methyl-(4*H*)-pyridazin-3-ones (3) A mixture of acid **2** (0.02 mol) and hydrazine hydrate (1.0 g, 0.02 mol) in ethanol (50 ml) was refluxed for 2 h. Then the mixture was cooled and the crude product which separated was filtered off and recrystallized from ethanol.

Ethyl 3-Oxo-5-substituted Benzylidene-6-methyl-(4*H*)-2-pyridazinylacetates (4a—d) The appropriate pyridazinones **3** (0.02 mol) were added to an ethanolic solution (50 ml) of sodium (0.46 g, 0.02 g atom). The mixture was refluxed for 30 min. Then, ethyl bromoacetate (3.34 g, 0.02 mol) was added by drops to the cooled solution, which was refluxed for 24 h and evaporated *in vacuo*. The residue was triturated with diisopropyl ether, and the solid which was formed was collected by filtration and dried. Compounds **4a—d** were recrystallized from a mixture of ethanol–water (50 : 50).

3-Oxo-5-substituted Benzylidene-6-methyl-(4*H*)-2-pyridazinylacetamides (5a—d) A suspension of compound **4** (0.007 mol) in ammonia (15 ml) and ethanol (5 ml) was heated in a bomb apparatus at 100 °C for 24 h. After cooling, the precipitate was filtered off and recrystallized from a mixture of ethanol–water (30 : 70).

3-Oxo-5-substituted Benzylidene-6-methyl-(4*H*)-2-pyridazinylacetohydrazides (6a—d) Hydrazine hydrate (15.0 g, 0.3 mol) was added to a solution of pyridazinone **4** (0.01 mol) in ethanol (15 ml). The solution was refluxed for 4 h. After cooling, the precipitate which separated was filtered off and recrystallized from ethanol.

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

Toxicity Behavioral Effects and Acute Toxicity: The compounds were administered at various doses. The animals were observed over 24 h and the symptomatology was noted. In addition, they were kept under observation for 8 d to detect any sign of toxicity.

Neurotoxicity: The rotarod test[17] 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 1 min.

Sedative Activity This activity was evaluated by a determination of the spontaneous motor activity. This test was performed by the method of Boissier and Simon[18] 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.

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

Effect on Maximal Electroshock Seizures[19]: 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 hindlimb tonic extensor component of seizures.

Effect on Seizures Induced by Pentylenetetrazole[19,20]: Test compounds were administered orally 30 min before evaluation for their ability to prevent the tonic extensor component induced by 85 mg/kg s.c. of pentylenetetrazole. Anticonvulsant activity was judged when the component was blocked.

Effect on Seizures Induced by Bicuculline[21]: The antagonism of bicuculline-induced seizures and lethality was evaluated according to a procedure similar to that described by Heyer. Compounds were administered 30 min before the test. Bicuculline was administered subcutaneously at a dose of 0.68 mg/kg. Protection against clonic and tonic seizures and against lethality was recorded.

Effect on Seizures Induced by Strychnine[22]: The ability of the test compound to provide a protection against seizures was measured 30 min after administration. Protection was defined as the abolition of the hind-leg tonic extensor component of the seizure induced by a 1.5 mg/kg s.c. injection of strychnine.

Effect on Tremors Induced by Oxotremorine[23]: Each test compound was given orally 30 min before administration of oxotremorine 0.5 mg/kg i.p. Half an hour after the injection of oxotremorine, each mouse was observed to evaluate tremor severity according to a rating scale with scores of 0, 1, 2 and 3. Intensity of tear secretion and salivation was also observed. Average suppression of the tremor by each dose of test compound was expressed as a percentage of inhibition of the control.

Sixty min after the injection of oxotremorine, rectal temperature was measured with a Carrieri thermorapid apparatus.

Lipophilicity Measurements The relative lipophilicity of the compounds was evaluated by reversed-phase TLC according to the procedure described in the literature.[24] Silica gel plates Merck RP-18 $F_{254}S$ were used as a nonpolar stationary phase. The plates were dried at 120 °C for 15 min before use. The polar mobile phase was an 80:20 (v/v) mixture of methanol and water. Each compound was dissolved in acetone (3 mg/ml), and 5 μl of the solution was applied onto the plate. The R_M values were calculated from the experimental R_F values according to the formula $R_M = \log[(1/R_F) - 1]$. Higher R_M values indicate higher lipophilicity.

Data Analysis Statistical analysis of the results was performed using the method of Schwartz.[25] The data on the spontaneous motor activity were analyzed by using the Student's *t*-test. All values were expressed as mean ± S.E. The data of pentylenetetrazole, bicuculline-, strychnine-induced seizures and oxotremorine-induced tremor were analyzed by means of the chi-square test with Yates correction. The ED_{50} were determined using the method of Litchfield and Wilcoxon.[26]

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References

1) A. Chimirri, A. de Sarro, G. de Sarro, S. Grasso, G. R. Trimarchi and M. Zappala, *J. Med. Chem.*, **32**, 93 (1989).
2) R. Sarges, H. R. Howard, B. K. Koe and A. Weissman, *J. Med. Chem.*, **32**, 437 (1989).
3) W. J. Brouillette, G. L. Grunervald, G. B. Brown, T. M. de Lorey, M. S. Akhtar and G. Liang, *J. Med. Chem.*, **32**, 1577 (1989).
4) M. J. Kornet and K. Filo, *Eur. J. Chem.*, **24**, 453 (1989).
5) S. M. Nanavati and R. B. Silverman, *J. Med. Chem.*, **32**, 2413 (1989).
6) M. R. Pavia, C. P. Taylor, F. M. Hershenson, S. J. Lobbestael and D. E. Butler, *J. Med. Chem.*, **31**, 841 (1988).
7) G. A. B. Davies-Jones, "Meyler's Side Effects of Drugs. Encyclopedia of Adverse Reactions and Interactions," ed. by M. N. G. Dukes Elsevier, Amsterdam, 1988, p. 120.
8) F. D. Popp, "Anticonvulsants," ed. by J. A. Vida, Academic Press, New York, 1977, p. 331.
9) I. Nitelea, M. Petrovanu, E. Rucinschi, M. Caprosu, I. Ciocoiu, E. Stefanescu, A. Carstea and I. Mihalcea, Rom. RO 75836 (28 Feb. 1981) [*Chem. Abstr.*, **99**, 139959*g* (1983)].
10) I. Nitelea, M. Petrovanu, E. Rucinschi, M. Caprosu, I. Ciocoiu, E. Stefanescu, A. Carstea and I. Mihalcea, Rom. RO 75837 (28 Feb. 1981) [*Chem. Abstr.*, **99**, 139960*a* (1983)].
11) P. Coudert, C. Rubat, J. Couquelet, J. Fialip and P. Bastide, *Eur. J. Med. Chem.*, **24**, 551 (1989).
12) J. M. Couquelet, J. Taoufik, J. D. Couquelet, P. Tronche, P. H. Chanh, A. P. H. Chanh, C. Navarro-Delmasure and B. Lasserre, *Eru. J. Med. Chem.*, **18**, 301 (1983).
13) J. Taoufik, J. D. Couquelet, J. M. Couquelet and A. Carpy, *J. Heterocyclic Chem.*, **21**, 305 (1984).
14) J. Taoufik, J. M. Couquelet, J. D. Couquelet and P. Tronche, *J. Heterocyclic Chem.*, **22**, 1615 (1985).
15) J. Taoufik, Thèse pour le Doctorat d'Etat ès Sciences Pharmaceutiques, *Ed. Bloc-Santé*, **194** (1982).
16) C. G. Wermuth, J. J. Bourguignon, G. Schlewer, J. P. Gies, A. Schoenfelder, A. Meukian, M. J. Bouchet, D. Chantreux, J. C. Molimard, M. Heaulme, J. P. Chambon and K. Biziere, *J. Med. Chem.*, **30**, 239 (1987).
17) W. J. Kinnard and C. J. Carr, *J. Pharmacol. Exp. Ther.*, **121**, 354 (1957).
18) J. R. Boissier and P. Simon, *Arch. Inter. Pharmacodyn.*, **158**, 212 (1965).
19) R. L. Krall, J. K. Peury, B. G. White, M. J. Kupferberg and E. A. Swinyard, *Epilepsia*, **19**, 409 (1978).
20) E. A. Swinyard, W. C. Brown and L. S. Goodman, *J. Pharmacol. Exp. Ther.*, **106**, 319 (1952).
21) E. Heyer, *Neurology*, **31**, 1381 (1981).
22) C. Gouret and P. Linee, *J. Pharmacol.* (Paris), **3**, 57 (1972).
23) C. Gouret, *J. Pharmacol.* (Paris), **4**, 105 (1973).
24) E. Tomlison, *J. Chromatogr.*, **113**, 1 (1975).
25) D. Schwartz, "*Méthodes statistiques à l'Usage des Médecins et des Biologistes*," ed. by Médicales Flammarion, 1984, p. 263.
26) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).