Pergamon

Bioorganic & Medicinal Chemistry, Vol. 2, No. 8, pp. 793-798, 1994

0968-0896/94 $7.00 + .00

0968-0896(94)00075-1

Synthesis, Anticonvulsant and Neuroprotective Activities of RP 66055, a Riluzole Derivative[1,*]

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Abstract—RP 66055, a riluzole derivative, has been characterized as a potent anticonvulsant and *in vivo* neuroprotective agent.

Introduction

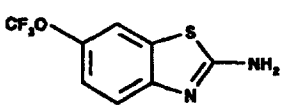
Riluzole 1 (6-trifluoromethoxy-2-benzothiazolamine, RP 54274, PK 26124) is a potent antagonist of excitatory amino acid-mediated (e.a.a.) neurotransmission,[2] a powerful neuroprotective agent,[3] and more recently riluzole appears to slow the progression of amyotrophic lateral sclerosis.[4] Riluzole is particularly efficacious at blocking convulsions induced by intracerebroventricular (icv) injection of e.a.a., such as L-glutamic acid.[2] Although this profile is only shared by antagonists of e.a.a. receptors (e.g. MK 801, NBQX, 7-chlorokynurenic acid),[5] riluzole does not appear to interact with any of the three major subtypes of e.a.a. receptors.[6] The 'antiglutamate' activity of riluzole may involve a modulation of L-glutamic acid release,[7] and the inactivation of voltage-dependent sodium channels.[8]

Consequently, the development of a comprehensive series of analogs, aimed at the identification of a second generation of potent anti-ischaemic agents, led us to choose the antagonism of L-glutamic acid-evoked convulsions in rats as a functional assessment of antiglutamate activity.

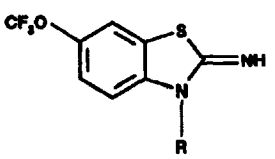
Following the discovery of the crucial importance of the 6-substituent in the benzothiazole series,[9] we went on to study the effect of the 3-substitution of riluzole itself. The synthesis of various 3-substituted-6-trifluoromethoxy-2-imino-benzothiazolines **2**[10] showed that strong protective effects against L-glutamic acid-induced seizures required the presence of 3-substituents containing either an unchanged or oxidized sulfur atom[11] or a nitrogen atom.

Optimization of the latter series of compounds (see Table 1) afforded **3a** which exhibits a significant protective effect against L-glutamic acid-induced seizures. In the hope of improving this anticonvulsant activity, substituents on the phenyl ring of the 4-phenyl piperazinyl chain of **3a** were introduced. In this series, the most active compounds bear in the 4-position only either a fluorine atom (**3c**, RP 66055) or a methoxy group (**3e**).[12] The 4-position seems to be of crucial importance for inhibition of the L-glutamic acid-induced seizures while introduction of a fluorine atom in the 2- or 3-position decreases the anticonvulsant activity.

In the present paper we report on the large-scale synthesis of RP 66055 and its anticonvulsant and neuroprotective activities.

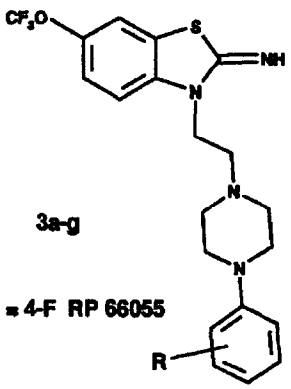


riluzole 1



2 R = $(CH_2)_nNR_1R_2$

R = $(CH_2)_nS(O)_mR_3$



3a-g

3c R = 4-F RP 66055

*Dedicated to Professor H. G. Viehe on the occasion of his 65th birthday

Results and Discussion

Synthesis

RP 66055 was prepared in a four-step process starting from 4-trifluoromethoxy-aniline **4** shown in Scheme I. Thus, acylation of **4** with chloracetyl chloride in the presence of triethylamine followed by reaction of crude **5** with 4-fluorophenyl-piperazine gave the amide **6** with an 86 % overall yield. Lithium aluminium hydride reduction of **6** in refluxing THF gave **7** in a 92 % yield. Benzothiazoline ring formation occurred under bromine/potassium thiocyanate reaction conditions in acetic acid to provide RP 66055 as the dihydrochloride salt with a 72 % yield after recrystallization. Using this synthetic pathway, a batch of 280 g of RP 66055 was prepared with a 57 % overall yield and without any chromatographic purification. Spetroscopic properties of compounds **6**, **7** and RP 66055 are listed in Table 2.

Binding studies

Binding assays have only shown an affinity of RP 66055 for voltage-sensitive sodium channels ([^{3}H]-BTX, IC_{50} = 0.4 μM). No affinity for Ca^{2+} channel, α1 and α2 adrenergic, 5-HT_2 serotonin, 5-HT uptake, D_2 dopamine, H_1 histamine, M1 muscarinic, AMPA, strychnine-insensitive glycine, NMDA-TCP and BZD receptors was observed (inactive at 0.1 to 10 μM).

RP 66055 displayed about a 100-fold higher potency for the voltage-sensitive sodium channels than riluzole ([^{3}H]-BTX, IC_{50} = 60 μM).

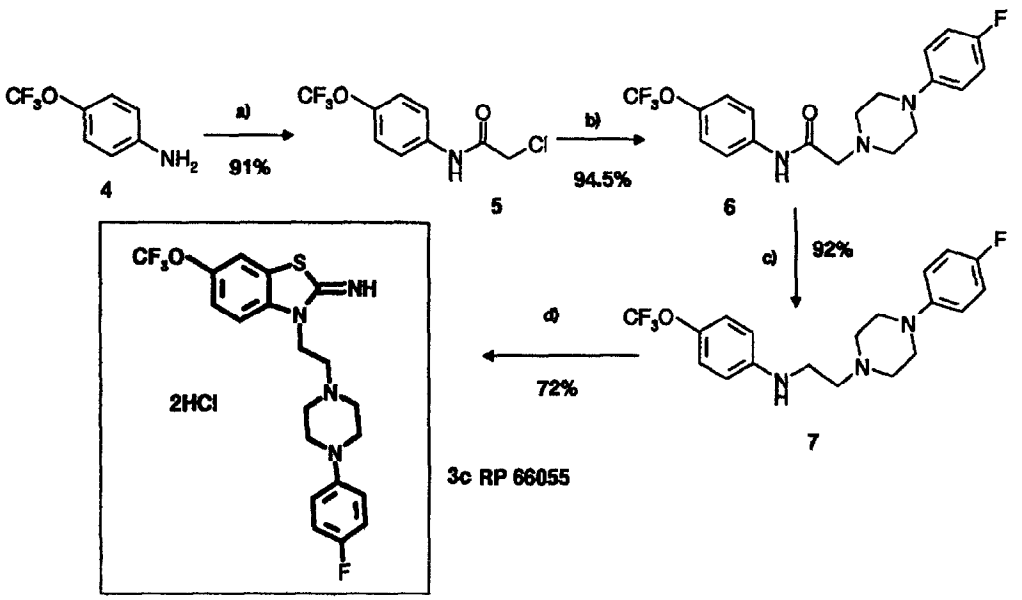
Sodium channel blockade of RP 66055

Given that RP 66055 was inactive in binding assays for the excitatory amino acid receptors, as shown above, suggests that its *in vivo* antiglutamate effect (see Table 1) cannot be explained by a direct interaction with these receptors. The absence of binding results and the high affinity for voltage-sensitive sodium channels ([^{3}H]-BTX) led us to envisage a modulation of L-glutamic acid release by inactivation of sodium channels. Consequently, we evaluated functionally the effects of RP 66055 on voltage-gated sodium channels by measuring the antagonism of veratridine-evoked dopamine release in rat striatal slices. RP 66055 exhibits a very potent antagonist activity: IC_{50} = 0.6 μM, compared with 3.5 μM for riluzole. Thus, there seems to be some relation between the *in vivo* antagonism of L-glutamic acid and the *in vitro* blockade of voltage-dependent sodium channels.

Table 1. Antagonism of L-glutamic acid-induced convulsions by compounds **3a–g** and riluzole

entry	R	ED_{50}[a] mg/kg i.p.	entry	R	ED_{50}[a] mg/kg i.p
a	H	3.5	e	4-OCH_3	2.5
b	4-Cl	5	f	2-F	6.2
c	4-F RP 66055	2.2	g	3-F	8.5
d	4-CH_3	10	riluzole		3.2

a) L-glutamic acid (12.5 μM/kg) was administered (10 μL/rat) intracerebroventricularly (icv). Compounds were administered intraperitoneally 30 min prior to challenge. After icv injection of L-glutamic acid, rats exhibited clonic seizures. The ED_{50} values (mg/kg) was defined as the dose of drug which protected 50 % of rats from convulsions.



Reagents and reaction conditions: a) $ClCH_2COCl$, $(C_2H_5)_3N$; THF, room temperature, 3 h; b) 4-fluorophenyl-piperazine, $NaHCO_3$, DMF, 80 °C, 12 h; c) $LiAlH_4$, THF, reflux, 3 h; d) Br_2, KSCN, AcOH, room temperature, 3 h, then HCl.

Scheme I. Synthesis of RP 66055

Table 2. Spectroscopic data of compounds **6**, **7** and RP 66055

Compound	IR $\nu_{max.}$ cm^{-1}	^{1}H-NMR.[a] δ [ppm, J(Hz)]
6	3325 (NH); 2820 (CH_2); 1705 (C=O); 1515(NH);1510 (aromatic ring);1265, 1225, 1205 and 1170 (O-CF_3)	(200 MHz) δ: 2.82 (m, 4H, NCH_2); 3.20 (m, 4H, $ArNCH_2$), 3.25 (s, 2H, $COCH_2$), 6.8-7.1 (m, 4H, Arom.), 7.22 (d, J = 8.5, 2H, Arom.), 7.63 (d, J = 8.5, 2H, Arom.)
7	3400 (NH); 2820 (CH_2); 1605 and 1505 (aromatic ring); 1265, 1225, 1195 and 1150 (O-CF_3); 825	(250 MHz) δ: 2.65 (m, 4H, NCH_2), 2.72 (t, J = 6.5, 2H, NCH_2), 3.15 (m, 4H, $ArNCH_2$), 3.20 (t, J = 6.5, 2H, $NHCH_2$), 6.61 (d, J = 8.5, 2H, H Arom.), 6.8-7.2 (m, 5H, Arom.)
RP 66055	3250 and 2750 (NH^+); 2600 and 2000 (NH^+); 1650 (C=N); 1585, 1515 and 1490 (aromatic ring), 1265, 1225, 1205 and 1175 (O-CF_3)	(200 MHz) δ: 3.2-3.8 (m, 10H, NCH_2), 4.9 (m, 2H, benzothiazole-NCH_2), 6.8-7.2 (m, 4H, Arom.), 7.60 (brd, J = 8.5, 1H, Arom.), 8.17 (br, 1H, Arom.), 8.19 (d, J = 8.5, 1H, Arom.),10-12 (br, 3H, NH and 2 HCl)

a) The ^{1}H NMR spectra were recorded in $CDCl_3$ (**6** and **7**) and in DMSO-d^6 (RP 66055); abbreviations used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet(s); br, broad signal.

Anticonvulsant activities

Like riluzole (ED_{50} = 3.2 mg/kg ip in rats),[2] RP 66055 (see Table 3) shows potent anticonvulsant properties against L-glutamic acid-induced convulsions (ED_{50} = 2.2 mg/kg ip in rats) and maximal electroshock (SME, ED_{50} = 2.3 mg/kg ip in mice). RP 66055 possesses an efficient CNS penetration since 10 min after iv administration it protects against SME seizures in rats with an ED_{50} of 23 µg/kg. Furthermore, RP 66055 displays a long-lasting effect after oral administration; its ED_{50} values at 16 h are only 5 or 9 times higher than those at 1 h (L-glutamic acid: ED_{50}s = 20 and 3.85 mg/kg po, respectively; SME: ED_{50} = 6 and 0.66 mg/kg po, respectively).

Given both on the one hand the potent *in vitro* blockade of voltage-dependent sodium channels and on the other hand the anticonvulsant activity, we studied the neuroprotective properties of RP 66055.

Neuroprotective properties of RP 66055

Hypobaric hypoxia in mice. Protective effects of RP 66055 against cerebral hypoxia were assessed using the following test:[13] mice were brought to a simulated altitude of 12000 m at a speed of 70 m/s (maximum hypobaric pressure = 200 hectopascals). After the simulated altitude was reached, the survival time for each mouse was measured. RP 66055 was given ip 30 min before the test.

RP 66055 produced a dose-dependent protection against mortality induced by hypoxia with a high efficacy (ED_{50} = 1.36 mg/kg ip). This result encouraged further study of the effects of RP 66055 on ischaemia-induced brain damage using both global and focal ischaemia models.

Bilateral carotid occlusion (BCO) in gerbils: a model of global and transient ischaemia. The ultimate goal of anti-ischaemic drugs is to reduce the extent of permanent brain tissue damage. Models of global ischaemia, such as transient bilateral carotid occlusion (BCO) in gerbils,[3a,14] attempt to reproduce the cessation of cerebral blood flow caused by cardiac arrest.

For BCO, gerbils were implanted with electrodes in the right parietal and occipital regions of the cranium. One week after this surgery they were anaesthetized with halothane and both carotid arteries were exposed and clipped for 6 min. RP 66055 (8 mg/kg) or vehicle was injected ip 0.5, 4.5, 24.5 and 28.5 h *after* release of carotid artery occlusion. On the fifteenth day post occlusion, the gerbils were decapitated and serial coronal brain sections were cut and stained with cresyl violet for histological examination. Brain muscarinic cholinergic receptor distribution in the same animals was studied by quantitative autoradiography using [^{3}H]-QNB binding.

RP 66055 (4 × 8 mg/kg ip over 28.5 h post-occlusion) significantly reduced necrosis in the hippocampal CA1 area induced by transient ischaemia. In this treated group, the cresyl violet coloration in the hippocampal CA1 area did not differ markedly from that observed in the sham-operated animals. Compared to sham gerbils, in untreated ischaemic gerbils a large decrease in muscarinic receptors was observed in the CA1 area (see Table 4). When RP 66055

Table 3. Anticonvulsant activities of RP 66055

Test[a]	Species	ED_{50} mg/kg
Glutamic acid-evoked convulsions	rat	2.2 ip
Maximal electroshock	mouse	2.3 ip 3.1 po 1.6 i.v.
Maximal electroshock	rat	0.66 po 0.023 i.v.

a) L-glutamic acid, maximal electroshock (50 mA, 50 Hz, 0.2 s) induced generalized clonic seizures. RP 66055 was administered prior to the test (10 min to 90 min). The ED_{50} was defined as the dose of RP 66055 which protected 50 % of animals from convulsions.

was given to gerbils after BCO, the decrease in [^{3}H]-QNB binding sites in CA1 area was attenuated by the drug (54 % $p<0.05$ %). These data agree with the histological studies referred to above.

Middle cerebral artery occlusion (MCAO) in rats: a model of focal and permanent ischaemia. Focal ischaemia[3b,15] in the rat caused by a permanent occlusion of the middle cerebral artery (MCA) has similarities to clinical thromboembolic stroke, although reperfusion may also be seen after stroke. Male Fisher rats were anaesthetized by halothane inhalation, and left middle cerebral artery occlusion was performed by the subtemporal approach. Seventy-two hours after occlusion, serial coronal sections of the brain were prepared and stained with cresyl violet for measurement of infarct volume. RP 66055 or vehicle was administered 0.5 and 24.5 h *after* MCAO. RP 66055 was administered at a dose of 8 mg/kg ip, or 0.5 or 1 mg/kg as a bolus iv.

In MCAO experiments, the size of the infarct was inversely related to the dose of RP 66055 (see Table 5). At a dose of 2 × 8 mg/kg ip, RP 66055 reduced by 44 % ($p < 0.001$) the infarct volume of the cortex. When delivered either at 2 × 1 or 2 × 0.5 mg/kg as a bolus iv, it significantly reduced the size of the cortex lesion by 31 % and 24 % respectively ($p < 0.05$).

These results demonstrate that RP 66055 has neuroprotective activity, both in transient ischaemia in gerbils (4 × 8 mg/kg ip) and in focal ischaemia in rats (2 × 8 mg/kg ip and 2 × 0.5 mg/kg iv). Furthermore, this study shows that even at the low dose of 0.5 mg/kg iv, RP 66055 reduces the size of infarct in a focal ischaemia model, even when administered 30 min after the onset of ischaemia.

In conclusion, RP 66055 showed potent anticonvulsant properties against L-glutamic acid-evoked convulsions and maximal electroshock in rats or mice with a long duration of action. RP 66055 is a very potent neuroprotective agent. It was active in hypobaric hypoxia in mice, and after curative treatments it possessed significant activity in two models of ischaemia (BCO and MCAO). A sodium blocking action of RP 66055 could explain, at least partially, both its anticonvulsant and anti-ischaemic properties. The very interesting pharmacological activity of RP 66055 should incite further electrophysiological studies in order to study its mechanism of action in depth.

Experimental

Chemistry

Commercially available reagents were used as received from suppliers. Solvents (THF, diethyl ether) were dried over 4 Å molecular sieves. The progress of the reactions was monitored by TLC on silica gel (Merck Kieselgel $60F_{254}$). Melting points were determined using a Reicher–Kofler apparatus and are uncorrected. ^{1}H NMR spectra were recorded on AC-200 or AC-250 Brücker spectrometers. IR spectra were recorded on a 983G Perkin–Elmer spectrophotometer or a 60SXR Nicolet spectrophotometer. Mass spectra were obtained on a Finnigan quadrupolar 3000 apparatus (EI; 70 eV) or on a Nermag R10-10B (DCI, reactant gas: NH_3). Elemental analyses were performed at Centre de Recherche de Vitry-Alfortville (Rhône-Poulenc Rorer).

N-*(Chloroacetyl)-4-trifluoromethoxyaniline 5*

To a solution of **4** (176 g, 1 mol) in THF (1.1 L) at ambient temperature under a nitrogen atmosphere was added dropwise and successively a solution of triethylamine (100 g, 143 mL, 1 mol) in THF (100 mL) and a solution of chloroacetyl chloride (114 g, 82 mL, 1 mol) in THF (400 mL). The stirring was then continued for 3 h at room temperature. The reaction mixture was cooled down with an

Table 4. Effects of RP 66055 on hippocampal neuronal damage in the BCO model. Autoradiographic quantification[a] of [^{3}H]-QNB binding sites in the gerbil hippocampal formation (pmol/mg protein)

Structures	Sham n = 4	Ischaemic n = 10	Ischaemic + RP 66055 n = 8
CA1	4.33 0.20	3.66 0.16*	4.02 0.18 [b]
CA3	3.99 0.16	3.88 0.09	4.14 0.14
Dg	5.34 0.13	5.19 0.09	5.30 0.20

a) Binding densities were determined from autoradiograms. Values represent the means of n animals ± SEM. Statistical analysis was performed using 1-way analysis of variance followed by Student's test. * $p < 0.05$: significant loss of CA1 pyramidal cells in the ischaemic preparation *vs* sham preparation. b) $p < 0.05$: significant reduced necrosis of CA1 pyramidal cells in the ischaemic preparation after RP 66055 *vs* ischaemic non-treated animals. CA1: CA1 field of the hippocampus, CA3: CA3 field of the hippocampus, Dg: Dentate gyrus.

Table 5. Effects of RP 66055 in the MCAO model. Infarct size 72 h after middle cerebral artery occlusion

	Dose mg/kg	Route	Number of rats	Cortex mm^3	Caudate nucleus mm^3	Total mm^3
Control	0	i.v. bolus	15	122.0 10.5	16.5 1.8	138.5 11.2
RP 66055	8	ip	12	68.0 10.5***	14.6 1.7	82.7 10.8**
RP 66055	1	i.v. bolus	14	84.5 9.1*	13.2 1.6	99.7 9.5*
RP 66055	0.5	i.v. bolus	12	92.7 8.7*	16.0 2.0	108.7 9.6

a) Infarct size (mm^3) 72 h after MCAO in the cortex and caudate nucleus. Data represent mean ± SEM. The areas of brain damage were measured on an image analyser. ***: $p < 0.001$; **: $p < 0.01$; *: $p < 0.05$ Dunnett's test.

ice bath, and the precipitate which formed was collected by filtration. The brown organic solution was dried over anhydrous magnesium sulphate and the solvent was removed on a rotary evaporator. The crude product was triturated with hexane (1 L) to give 230 g (91 %) of **5** as pale yellow-coloured solid (mp 130 °C, R_f = 0.6 in ethyl acetate–cyclohexane mixture 3:7), which was used for the next step without further purification. Mass spectrum (EI) m/z: 253 ($M^{+\cdot}$), 204 (M-CH_2Cl), 177 (M-$COCH_2Cl$), 108 (177-CF_3, 100 %).

N-(4-Trifluoromethoxyanilino)-2-[1-(4-fluorophenyl-piperazinyl)]acetamide **6**. To a solution of **5** (230 g, 0.91 mol) in DMF (2.2 L) was added in small portions at room temperature $NaHCO_3$ (77.3 g, 0.92 mol) and then dropwise a solution of 4-fluorophenyl-piperazine (167 g, 0.93 mol) in DMF (500 mL) under nitrogen. The solution was stirred for 12 h at 80 °C. After cooling, the DMF was removed to afford a brown residue which was triturated with a water-diethyl ether mixture 1:1 (500 mL). The organic phase was dried over anhydrous magnesium sulphate and concentrated to give crude **6** (360 g) which was recrystallized from hexane (1 L) to afford pure **6** as a cream solid (341 g, 94.5 %, mp 84 °C, R_f = 0.35 in ethyl acetate–cyclohexane mixture 1:1). Mass spectrum (EI) m/z: 397 ($M^{+\cdot}$); 193 (100 %).

N-{2-[1-(4-Fluorophenyl-piperazinyl)]ethyl}-4-trifluoro-methoxyaniline **7**. A solution of the acetamide **6** (341 g, 0.86 mol) in THF (1.5 L) was added dropwise over 1 h into a solution of lithium aluminium hydride (65.0 g, 1.72 mol) in THF (1 L) at such rate that the temperature did not rise above 40 °C under nitrogen. The mixture was refluxed for 3 h, then cooled down with an ice bath. The reaction mixture was quenched with *caution* with saturated aqueous NH_4Cl (850 mL) and diluted with water (1 L). The suspension was filtered, and the solution was extracted with diethyl ether. The organic layer was dried over anhydrous magnesium sulphate and concentrated to give 354 g of crude **7** as a cream-coloured solid. Pure **7** was obtained after recrystallization from hot hexane–diethyl ether mixture 7:1 as white solid (303 g, 92 %, mp 92 °C, R_f = 0.6 in diethyl ether–cyclohexane mixture 9:1). Mass spectrum (EI) m/z: 383 ($M^{+\cdot}$); 193 (100 %).

3-{2-[1-(4-Fluorophenyl-piperazinyl)]ethyl}-2-imino-6-trifluoromethoxybenzothiazoline, dihydrochloride RP 66055. Potassium thiocyanate (295 g, 3 mol) was added in small portions at room temperature into a solution of **7** (291 g, 0.76 mol) in glacial acetic acid (2.2 L), then stirring was continued for 30 min under a positive pressure of nitrogen. Bromine (128 g, 41 mL, 0.84 mol) in glacial acetic acid (800 mL) was then added dropwise at room temperature to this yellow solution. A light exothermic reaction was observed, a decoloration appeared within a few minutes, and a white solid appeared. The reaction mixture was stirred at room temperature for 3 h. The white solid was then filtered and the solution was concentrated to give 725 g of a brown solid which was diluted with water (500 mL), ethyl acetate (200 mL) and then basified with 10 N NaOH (100 mL). The organic layer was dried over anhydrous magnesium sulphate and concentrated to afford 334 g of crude RP 66055 as a brown oil which then was dissolved in ethanol (3 L). An etheral solution of hydrogen chloride (760 mL) was then added to precipitate most of the dihydrochloride of RP 66055 in the form of pale yellow needles [204.6 g, 52.5 %, mp 262 °C, R_f (free base) = 0.38 in ethyl acetate]. The solvent was removed *in vacuo* and some additional RP 66055 was obtained after crystallization from ethanol–water mixture 1:1 (75.4 g); overall yield of RP 66055: 280 g, 72 %. Mass spectrum (EI) m/z: 290 (M-$C_9H_{10}NF$ + H), 206, 150 (C_9H_9NF, 100 %), 70; (DCI) m/z: 441 (M + H^+, 100 %.). Anal. calcd for $C_{20}H_{22}Cl_2F_4N_4OS$: C, 46.79; H, 4.32; Cl, 13.81; F,14.8; N, 10.91; S, 6.25. Found (2 HCl): C, 47.0; H, 4.2; Cl, 14.0; F, 14.8; N, 10.7; S, 6.4.

Radioligand binding assay

All the radioligand assays were performed according to classical methods described in the literature. Only the binding of batrachotoxin ([^{3}H]-BTX) is given: the affinity for the batrachotoxin-sensitive sodium channel site was determined according to the method described by Pauwels *et al.*[16] with slight modifications. The homogenate of brain without cerebellum from female Sprague Dawley rats was used as source of receptors. Binding reactions were initiated by adding samples or solvent with 5 ± 0.5 nmol [^{3}H]-batrachotoxinin A 20α-benzoate (Du Pont de Nemours, NEN, Specific activity 1.11–2.22 TBq/mmol), 1 μM tetrodotoxin, 50 μg/mL of scorpion venom from *Leirus q. quinquestriatus* (Latoxan, France), 0.42 ± 0.04 mg/ mL of tissue homogenate in HEPES buffer 50 mM, pH 7.4 containing 130 mM choline chloride, 5.5 mM glucose, 0.8 mM $MgSO_4$, and 5.4 mM KCl. This mixture was incubated for 60 min at 37 °C. The non specific binding was obtained with 600 ± 60 μM veratridine. The binding was terminated by filtration across Whatman GF/B fibre filters with a Skatron cell harvester, followed by three washes with 4 mL of ice cold HEPES buffer 5 mM, pH 7.4 containing 163 mM choline chloride, 5.5 mM glucose, 1.8 mM $CaCl_2$, and 0.8 mM $MgSO_4$. The radioactivity retained on the filters was determined by liquid scintillometry in 4 mL Ready gel Beckman. The IC_{50} was determined from five concentrations in duplicate using a log-probit determination.

Veratridine-evoked dopamine release[17]

Male rats (Sprague Dawley, 200–350 g) were killed by decapitation and the striata were sliced in two dimensions (0.3 × 0.3 mm) to form ribbons, which were incubated for 15 min at 37 °C with [^{3}H]-dopamine (Amersham, Specific activity 1.74 TBq/mmol) at the final concentration of 50 nM in a modified Krebs medium (composition in mM: NaCl, 118; KCl, 5; $NaHCO_3$, 25; NaH_2PO_4, 1; $MgCl_2$, 1.2; $CaCl_2$, 1.9; glucose, 11.1; ascorbic acid, 0.1; pargyline, 0.01; bacitracine, 0.02) and serum albumin 0.1 %, continuously gassed with 95 % O_2/5 % CO_2. The tissue was then rinsed and aliquots containing approximately 15 mg tissue were transferred to a superfusion system using Millipore filters (Millex; 0.45 μM) as superfusion chambers. After a 30 min superfusion period at 0.4 mL/min, 2-min samples were collected

directly into vials and the radioactivity was measured by liquid scintillation spectrometry. Depolarization-induced increase in [^{3}H]-dopamine was accomplished by superfusion for 1 min with 5 μM veratridine, 8 min (4 fractions) after the beginning of the superfusion. RP 66055 was added 4 min before veratridine and maintained throughout the superfusion. The radioactivity remaining on the filter at the end of the experiment was measured and the fractional rate of [^{3}H]-dopamine release was expressed as a percentage of the radioactivity present in ribbons at the beginning of each fraction. The total overflow was calculated as the difference between i) the percent release induced by depolarization in all combined fractions and ii) the percent release in the combined fractions with no depolarization (baseline value). For each concentration, the percentage of antagonism was determined. Data were analysed using a nonlinear curve fitting program (Enzfitter) from which the concentration of compound inhibiting 50 % of the response to veratridine was calculated (IC_{50}).

Pharmacology

Test of anticonvulsant activity against L-glutamic acid induced seizures in rats. Groups of 7 male Sprague Dawley rats weighing about 200 g were used. Drugs were injected ip or po, 30 min or 1 h respectively, prior to intracerebroventricular (icv) administration of 10 μL/rat of a 12.5 μmol/kg solution of L-glutamic acid in saline according to a free hand technique. After this treatment, the rats were observed for 30 min; L-glutamate alone provoked seizures immediately after injection: animals ran wildly, jumped violently and exhibited clonic seizures. Results were expressed as all or none. ED_{50} for studied compounds was defined as the dose of drug (mg/kg) which totally protected 50 per cent of the rats from clonic convulsions and jumps.

Test of anticonvulsant activity against maximal electroshock induced seizures in mouse or rats. Anticonvulsant activity was evaluated in groups of 10 or 12 male mice (CD1 Charles River) or 8 male Fischer rats (Charles River) against tonic convulsions induced by SME, according to the method described by Swinyard *et al.*[18].

Tests of neuroprotective activities. Neuronal protection by RP 66055 against ischaemic brain damage in gerbils (BCO model) was evaluated according to the method described by Araki *et al.*,[14] and in rats (MCAO model) was performed as described by Tamura *et al.* [15]

Acknowledgements

We wish to thank N. Achaibou, F. Debarnot, G. Durand, P. Ganil, M. Meunier, J.-M. Miquet, M. Pasquet, C. Pauchet and M. Roux for technical assistance, and M. Vuilhorgne and co-workers for spectral analyses.

(*Received* 5 *May* 1994)

References and Notes

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