Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 1627–1637

BIOORGANIC & MEDICINAL CHEMISTRY

9-Carboxymethyl-5*H*,10*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one-2-carbocylic Acid (RPR117824): Selective Anticonvulsive and Neuroprotective AMPA Antagonist

Serge Mignani,* Georg Andrees Bohme, Guillaume Birraux,† Alain Boireau, Patrick Jimonet, Dominique Damour, Arielle Genevois-Borella, Marc-Williams Debono, Jeremy Pratt, Marc Vuilhorgne, Florence Wahl and Jean-Marie Stutzmann

Aventis Pharma S.A., Centre de Recherche de Paris, 13 quai Jules Guesde, B.P. 14, 94403 Vitry-sur-Seine Cedex, France

Received 9 October 2001; accepted 10 December 2001

Abstract—Excessive release of glutamate, a potent excitatory neurotransmitter, is thought to play an important role in a variety of acute and chronic neurological disorders, suggesting that excitatory amino acid antagonists may have broad therapeutic potential in neurology. Here, we describe the synthesis, pharmacological properties and neuroprotective activity of 9-carboxymethyl-imidazo-[1-2*a*]indeno[1-2*e*]pyrazin-4-one-2-carboxylic acid (**RPR117824**), an original selective AMPA antagonist. **RPR117824** can be obtained through a six-step synthesis starting from (1-oxo-indan-4-yl) acetic acid, which has been validated on a gram-scale with an overall yield of 25%. Monosodium or disodium salts of the compound exhibit excellent solubility in saline ($\geq$10 g/L), enabling intravenous administration. **RPR117824** displays nanomolar affinity (IC_{50} = 18 nM) for AMPA receptors and competitive inhibition of electrophysiological responses mediated by AMPA receptors heterologously expressed in *Xenopus* oocytes (K_B = 5 nM) and native receptors in rat brain slices (IC_{50} = 0.36 μM). In in vivo testing, **RPR117824** behaves as a powerful blocker of convulsions induced in mice or rats by supramaximal electroshock or chemoconvulsive agents such as pentylenetetrazole, bicuculline, isoniazide, strychnine, 4-aminopyridine and harmaline with half maximal effective doses ranging from 1.5 to 10 mg/kg following subcutaneous or intraperitoneal administration. In disease models in rats and gerbils, **RPR117824** possesses significant neuroprotective activity in global and focal cerebral ischemia, and brain and spinal cord trauma.

Introduction

Over the last 20 years, it has been well established that L-glutamate is the major fast excitatory neurotransmitter in the mammalian central nervous system (CNS). Glutamate plays an essential role in many physiological CNS functions through the activation of three major types of postsynaptic ionotropic receptors designated according to the non-natural substances that selectively activate them: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and Kainate receptors. In addition, glutamate activates several types of metabotropic receptors signalling through a G-protein coupled second messenger system.[1] AMPA receptors are composed of four subunits (GluR1–GluR4) that are widely, but differentially, expressed throughout the CNS and form functional receptors as heterotetrameric subunit assemblies.

The excessive release of glutamate is thought to partecipate in the pathophysiological cascade of a variety of neurological disorders, including cerebral ischemia following cardiac arrest, thromboembolic or hemorrhagic stroke, hypoglycemia, epilepsy, and cerebral or spinal cord trauma.[2] Glutamate might also contribute to the progression of chronic neurodegenerative diseases such as Alzheimer's and Parkinson's disease.[3] Consequently, AMPA antagonists may have an important therapeutic potential in the treatment of a variety of acute and chronic neurological diseases.

Heretofore described antagonists at AMPA receptors belong to the following chemical families:[4] (i) quinoxalines, (ii) heterocyclic-fused quinoxalinones, (iii) isatinoximes, (iv) quinazolines, (v) quinolones, (vi) decahydroisoquinolines and (vii) dihydroph-

*Corresponding author. Fax: +33-1-5571-8014; e-mail: serge.mignani@aventis.com

†Present address: ASTER, 3-5 rue Eugène-Millon, 75015 Paris, France.

0968-0896/02/$ - see front matter
PII: S0968-0896(01)00431-X

thalazines. Representative examples of these classes are **NBQX**,[5] **YM90K**,[6] **YM872**,[7] **NPQX**,[8] **(−)-LY 293558**[9] and **LY-300164**[10] (Fig. 1). To date, **YM-872** and **LY-300164** (talampanel) have been evaluated in clinical trials Phase I/II for cerebrovascular ischemia (Fig. 1).[11]

As part of our program directed towards the development of new excitatory amino acid antagonists based on the recently identified anticonvulsant and neuroprotective lead compound imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one **1**,[12] we prepared compounds with improved in vitro and in vivo activities. We first synthesized spiro-imidazo[1,2-*a*]indeno[1,2 - *e*]pyrazin - 4 - one derivatives such as **(+)-2**[13] which exhibited affinities for both the AMPA receptor and the glycine modulatory site of the NMDA receptor. We then identified antagonists with selectivity for AMPA receptors and good in vivo activity among 8-methylureido-10-carboxymethyl (-10-carboxylidene) imidazo[1,2 - *a*]indeno[1,2 - *e*]pyrazin-4-one derivatives such as **(+)-3a** and **3b**,[14] 8-methylureido-4-oxo-imidazo[1,2-*a*]indeno[1,2 - *e*]pyrazin - 2 - carboxylic acid derivatives such as **4**[15] and finally carboxyalkyl-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic (acetic) acid derivatives such as **5**.[16a,b] Here, we detail the synthesis and the pharmacological properties of a member of the latter family, namely 9-carboxymethyl-imidazo[1-2*a*]indeno[1 - 2*e*]pyrazin - 4 - one-2-carboxylic acid (**RPR117824**, Fig. 2). This compound exhibits nanomolar binding affinity and competitive antagonist properties at AMPA receptors, in vivo activity in convulsion models in the milligram dose-range and protective activity in animal models of acute neurological injury.

Results and Discussion

Chemistry

An optimized synthesis of **RPR117824** is outlined in Scheme 1. The indanone derivative **6**[16b] was converted into the corresponding bromo derivative **7** with bromide in glacial acetic acid (84% yield). Esterification of **7** with oxalyl chloride in methylene chloride and in the presence of ethanol gave **8** with a 53% yield. Then, condensation of **8** with 4-ethoxycarbonyl-imidazole-2-carboxylate **9**[17] using potassium carbonate as base in acetone at reflux gave **10** with a 82% yield. Finally, intramolecular ring closure of **10** with ammonium acetate in glacial acetic acid directly produced the cyclized derivative **11** (77% yield) which was readily saponified into the corresponding tris-sodium salt **12** through the action of 1 N sodium hydroxide with a 94% yield. The free acid **RPR117824** was obtained by acidification with 0.3 M hydrochloric acid (94% yield).

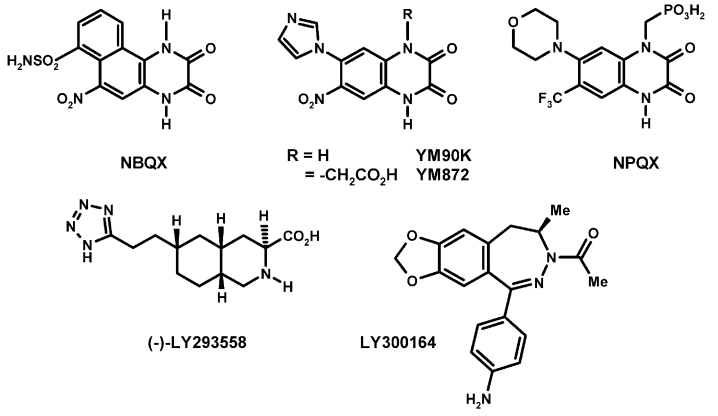


Figure 1.

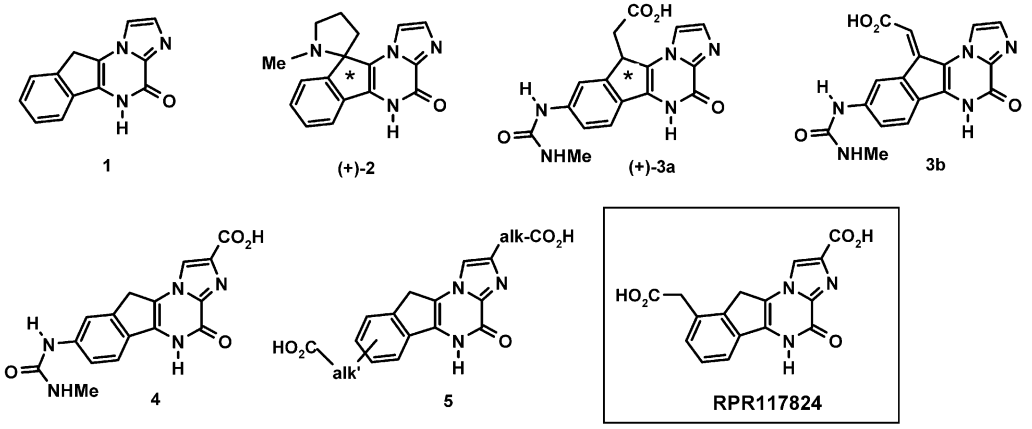


Figure 2.

Pharmacological activity

Binding studies. Affinities for AMPA receptors and for the glycine modulatory site pertaining to the NMDA receptor were evaluated in binding assays on rat cortical membrane preparations using [^{3}H]-AMPA and [^{3}H]-5,7-dichlorokynurenate ([^{3}H]-DCKA), respectively, as selective ligands. Mean data for compounds **1**, **RPR117824**, **NBQX**, **YM90K** and **(−)-LY293558** are given in Table 1. **RPR117824** displayed a ~40-fold higher potency than was previously observed for the unsubstituted parent compound **1**, and an 8- to 19-fold greater affinity with respect to the comparator standards **NBQX** and **YM90K**.

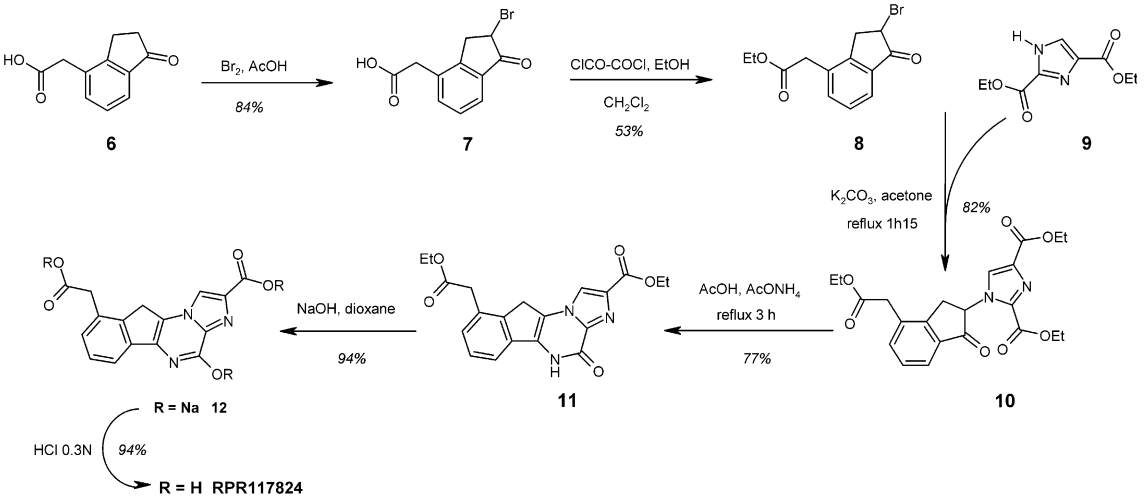
Functional studies. The intrinsic activity and mechanism of antagonist action of **RPR117824** were determined using *Xenopus* oocytes injected with mRNA extracted from rat cortex. **RPR117824** antagonized kainate-induced responses in a concentration-dependent manner. This antagonism appeared to be competitive as shown by a parallel rightward shift of the concentration–response curve to kainate in the presence of increasing concentrations of the compound without depression of maximal responses (Fig. 3A). Schild-plot analysis of this effect yielded a K_B value of 5 nM (Fig. 3B).

The effects of **RPR117824** at native glutamatergic synapses were examined on rat hippocampal slices maintained in vitro. The compounds under examination were applied through the superfusion bath surrounding the slices. Concentrations of **RPR117824** of 0.1 μM and

Table 1. In vitro pharmacology of **RPR117824**, **NBQX** and **YM90K**[a]

	Binding affinity		Antagonist activity[b]
	AMPA	NMDA	Oocytes
1	760	3000	1800
RPR117824	18	7200	5
NBQX	140	>10,000	31
YM90K	350	10,400	260

[a]IC_{50} values are mean of at least three determinations, each with at least three concentrations of tested compound in triplicate.
[b]IC_{50} values (except for **1** and **RPR117824** : K_B values in nM; **1** from ref 8) for inhibition of currents generated by 50 μM kainate in *Xenopus* oocytes injected with rat brain mRNA.



Scheme 1.

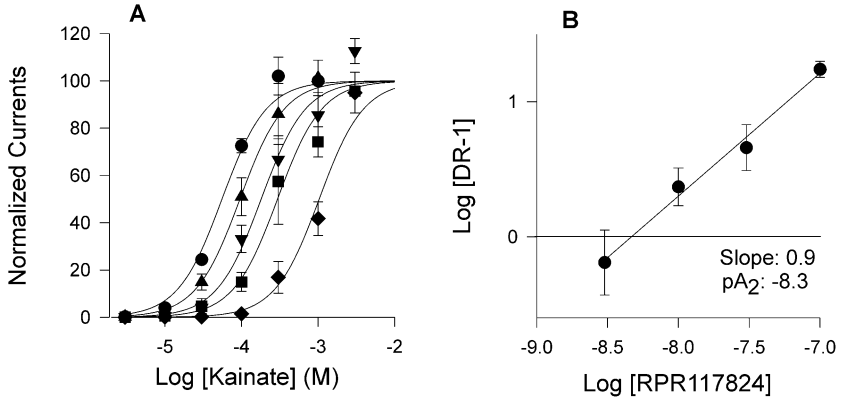


Figure 3. (A) Concentration–response curves to kainate in the presence of the indicated concentrations of **RPR117824**. Data are mean ± SEM from $n = 3$ cells. (B) pA_2 determination using the Schild plot analysis. The affinity constant was found by interpolating log (DR−1) to zero. ● = control; ▲ = 3 nM; ▼ = 10 nM; ■ = 30 nM; ◆ = 100 nM.

above depressed field EPSP amplitude in a concentration-dependent manner (Fig. 4). No reversal of this effect was observed upon washout with drug-free ACSF for up to 90 min after the start of **RPR117824** application. Figure 4 shows also the effect of **YM90K** examined in the same conditions. This compound similarly depressed hippocampal synaptic transmission but the threshold concentration for this drug was 3-fold higher than for **RPR117824** and a reversal of its effect was consistently observed at intermediate concentrations within 90 min of bath application, indicating a shorter duration of action. A sigmoidal curve fitted to the data by non-linear least square regression analysis indicated that **RPR 117824** was approximately 6-fold more potent than **YM90K** with IC_{50} of 0.36 compared to 2 μM, respectively.

Anticonvulsant activity. In order to examine the activity of **RPR117824** on the CNS in vivo, its effect against electrically-induced convulsions was tested in mice upon administration by various routes and with varying time intervals before challenge. The compound was not active by oral route. Results are illustrated in Table 2. Briefly, **RPR117824** is a potent anti-convulsant by parenteral routes in the electroshock test, with ED's of about 1 mg/kg ip or sc and an ED iv of 0.54 mg/kg; the ED_{50} being 25.6 mg/kg iv 3 h post administration.

As part of a more through evaluation of the anticonvulsant profile of **RPR117824**, its activity against six chemoconvulsant agents featuring different mechanisms of convulsant action was tested in comparison with **YM90K**. The GABA receptor blockers pentylenetetrazole (PTZ) and bicuculline, the glutamate decarboxylase inhibitor isoniazide, the glycine-site blocker strychnine, the potassium channel blocker 4-aminopyridine (4-AP) and the glutamate uptake inhibitor harmaline were used as chemoconvulsant substances. Table 3 summarizes the findings: **RPR117824** completely blocked generalized clonic seizures induced by PTZ, isoniazide, strychnine and 4-AP, and inhibited harmaline-induced tremor. However, it had only partial anticonvulsant activity against biccuculine. **YM90K** was inactive at 20 mg/kg or above in all tests except against isoniazide (ED_{50} = 19 mg/kg). It was not tested against strychnine.

Table 2. Anticonvulsant activity of **RPR117824** against supramaximal electroshock seizures in mice

Route of administration	Pretreatment time (min)	ED_{50} (mg/kg)
ip	30	0.83
sc	30	1.04
sc	60	2.0
sc	180	7.5
sc	360	18
iv	5	0.54
iv	30	0.95
iv	60	7.4
iv	180	25.6

Table 3. Anticonvulsant activity of **RPR117824** and **YM90K** against generalized clonic seizures induced by chemoconvulsants in mice

Convulsant agent	**RPR117824**	**YM90K**
Pentylentetrazole	1.7[a]	>20
Bicuculline	1.7[b]	>40
Isomiazide	1.5	19
Strychnine	6.4	(not tested)
4-Aminopyrine	16	>80
Harmaline[c]	9.8	>40

Data are ED_{50} values in mg/kg by sub-cutaneous route (unless otherwise specified).
[a]Against tonic hindpaw extension.
[b]By intraperitoneal route.
[c]Against tremor.

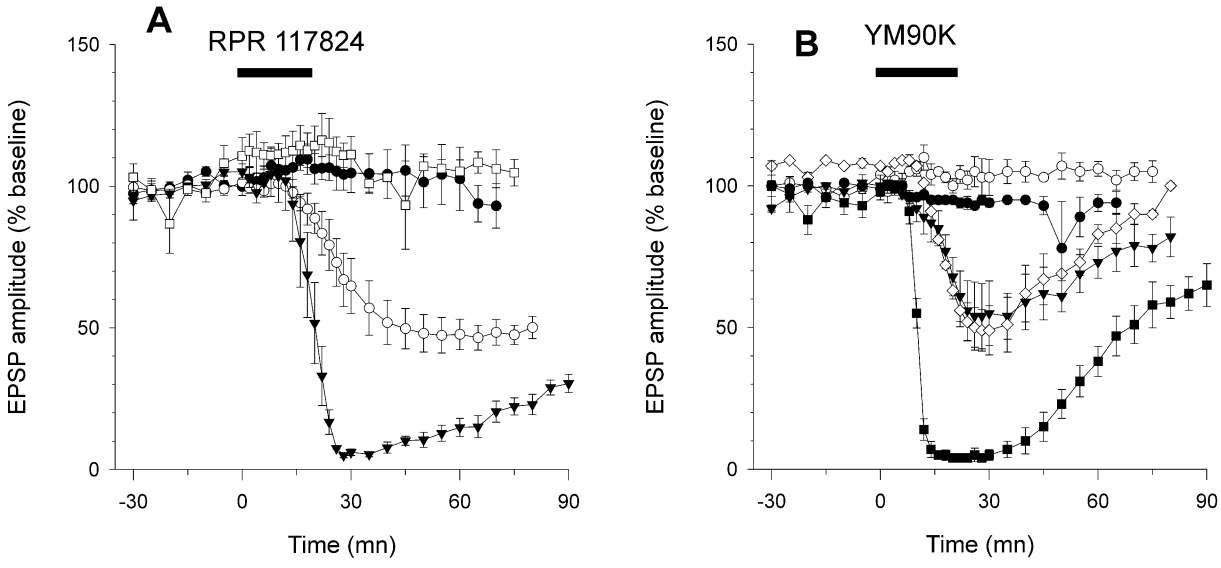


Figure 4. (A) Time-course of inhibitory effect of RPR 117824 on synaptic transmission in vitro. Mean (±SEM) change in normalized amplitude of CA1 hippocampal field EPSP in response to application of increasing concentrations of **RPR117824**. Bar above graph represents duration of application containing ACSF (not corrected for dead space of the perfusion system). □ = 0.03 μM; ● = 0.1 μM; ○ = 0.3 μM; ▼ = 1 μM (all n = 3). In B: Similar experiments with **YM90K.** ○ = 0.1 μM; ● = 0.3 μM; ▼ = 1 μM; ◇ = 3 μM; ■ = 10 μM (all n = 3). Note the shorter duration of action and the higher concentrations needed for inhibition with this compound.

Neuroprotective activity in disease models

Ischemia-induced brain damage. Occlusion of the two common carotid arteries of the gerbil (BCO) over 5 min provokes a global cerebral ischemia followed by reperfusion damage which has behavioral and structural consequences. These include a pronounced hypermotility, reduced performance in a learning/adaptation test and structural damage to the hippocampus. One week after the ischemic insult and drug treatments, **RPR117824** was able to significantly reduce hypermotility in the openfield from the dose of 4 mg/kg sc (Fig. 5A) and when treatment at 8 mg/kg sc was started up to 30 min post-ischemic insult (Fig. 5D). This compound had a significant protective effect on the learning/adaptation ability of the animals in a step-down paradigm when treatment was given either at 16 mg/kg 30 min post-ischemic insult (Fig. 5B), or when treatments were started at 8 mg/kg up to 3 h post insult (Fig. 5E). The pyramidal cell layer of the hippocampus was also significantly protected from the dose of 8 mg/kg sc started 30 min post ischemic insult (Fig. 5C and F). Protection from the deleterious effects of the ischemic insult is dose-dependent from the dose of 8 mg/kg when treatment is started 30 min post ischemic insult and starting the treatment is almost as effective 30 min post insult as 15 min preinsult. However, if treatment is delayed to 3 h post insult, beneficial effects are greatly reduced.

Permanent middle cerebral artery occlusion (pMCAO) in rats induced the development of cerebral lesions affecting both the cortex and the striatum. In this model, **RPR117824** at 2×16 mg/kg given 10 min post occlusion (iv) and 2 h after (sc), significantly reduced the cerebral lesions by 22% ($p<0.05$). This effect was only observed in the cortex (−27%, $p<0.05$; data not shown). In contrast **RPR117824** administered at a lower dosage (2×8 mg/kg) showed no effect. In a delayed administration **RPR117824** at 2×16 mg/kg given 30 min (iv) and 2 h 30 min (sc) post-ischemia onset significantly reduced cerebral lesions by 24% ($p<0.05$). This effect was also observed in the cortex (−25%, $p<0.05$; data not shown) (Fig. 6).

Trauma-induced alteration of brain and spinal cord function. RPR117824 was evaluated in a model of traumatic brain injury in rats. Fluid percussion of moderate severity (1.6–1.8 bar) was applied laterally to the right parietal cortex to induce lesions of the surrounding structures. **RPR117824** at 3×4 mg/kg administered 15 min (iv) 6 and 24 h (sc) post-TBI, significantly reduced

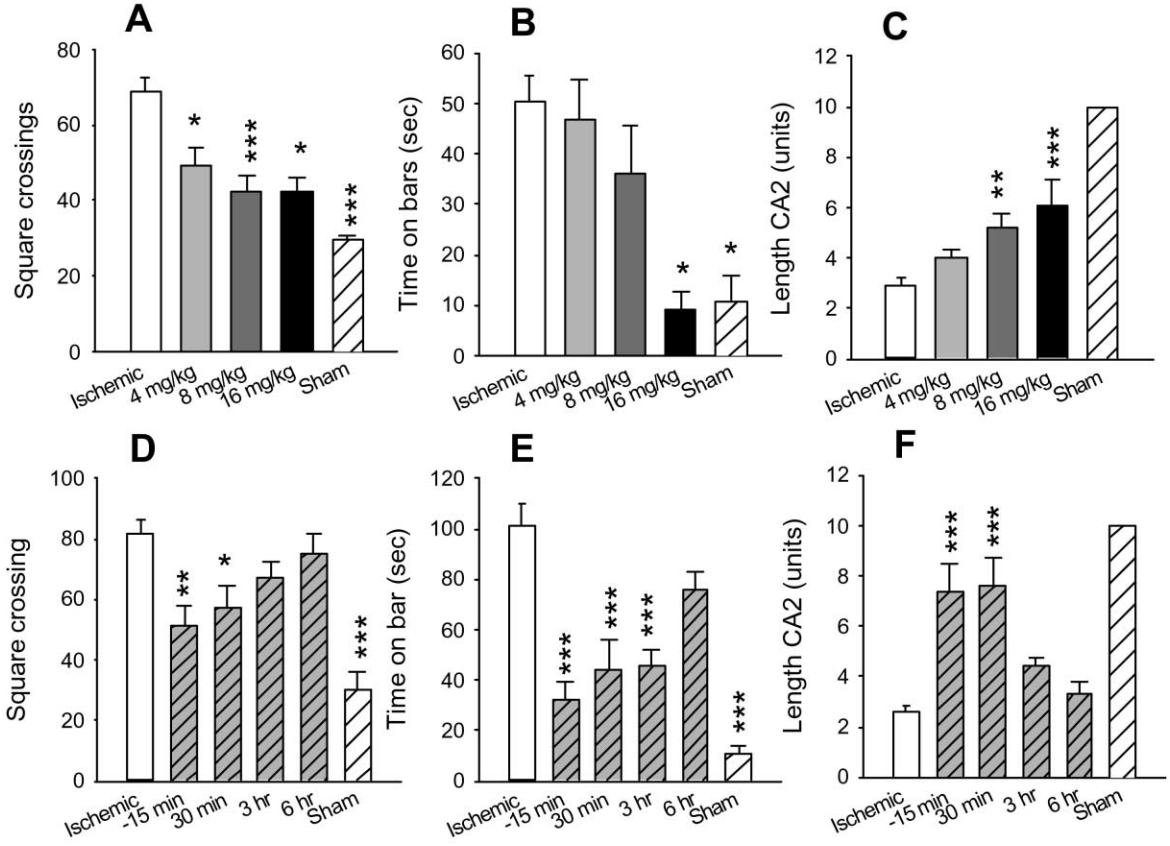


Figure 5. Gerbil BCO: On top line, dose–response results of treatment with **RPR117824** at 4, 8 or 16 mg/kg sc ($n=11$, 11, 5, respectively, with 25 vehicle-treated controls and nine sham-operated animals) On second line, the effects of starting treatment 15 min before, 30 min, 3 or 6 h post-ischemic insult are illustrated ($n=9$/treatment group against 14 controls and seven sham animals). Results in Graphs A and D show motility in an open field over 3 min. Graph B and E show passive avoidance learning as time spent on electrified bars over a 4-min period immediately following a 1-min learning period. Graph C and F show length of CA2 zone of hippocampus. All statistics ANOVA and Tukey–Kramer multiple comparison test compared with vehicle-treated controls.

the cortical lesion by 29% ($p < 0.05$). In contrast, at a higher dosage (3×8 mg/kg), no effect was observed (Fig. 7).

Compression of the spinal cord results in paralysis of the hind limbs. In the spinal cord injury (SCI) model in rats, **RPR117824** conferred a degree of protection, as illustrated by the increased ability of the animals to remain on an inclined plane requiring the angle of the inclined plane to be significantly greater in order to make the animals slide, the significant improvement in the neurological score and a general trend towards more normal behavior throughout was also noted (Fig. 8).

Conclusion

RPR117824, an original and selective AMPA antagonist, displays selective and potent affinity for the AMPA receptor with an IC_{50} of 18 nM and competitively inhibited functional responses mediated by these receptors. **RPR117824** showed a wide range of anticonvulsant activities in several in vivo models such as supramaximal electroshock test in mice and possessed anticonvulsant activity against chemoconvulsive agents: pentylenetetrazole, bicuculline, isoniazide, strychnine, 4-aminopyridine and harmaline with ED_{50}'s ranking between 1.5 and 10 mg/kg sc or ip in mice or rats. In addition, **RPR117824** exhibited neuroprotective properties, when administered post-insult, in acute neurodegenerative disease models of CNS ischemia and trauma such as the BCO in gerbils (global ischaemia) and MCAO in rats (focal ischaemia), and traumatic brain injury and spinal cord injury models in rats by iv/sc administration. **RPR117824** is an AMPA antagonist with interesting anti-convulsant and neuroprotective properties.

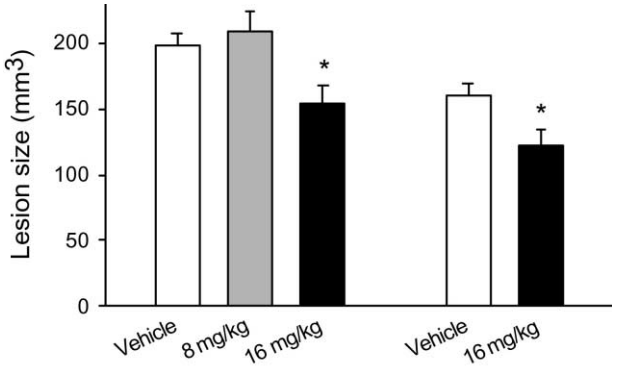


Figure 6. Rat pMCAO, volumes of brain lesions: **RPR117824** was administered either at 2×8 ($n = 13$) and 2×16 mg/kg ($n = 13$) given at 10 min iv and 2 h sc post-ischemia onset (left graph) or at 2×16 mg/kg ($n = 13$) given at 30 min iv and 2 h 30 min sc post-ischemia onset (right graph). Vehicle was administered following respective protocols ($n = 14$ in each study). Rats were killed and brains were removed 2 days post ischemia. Areas of cerebral lesion were measured after staining of coronal cryostat brain sections with 0.5% cresyl violet.

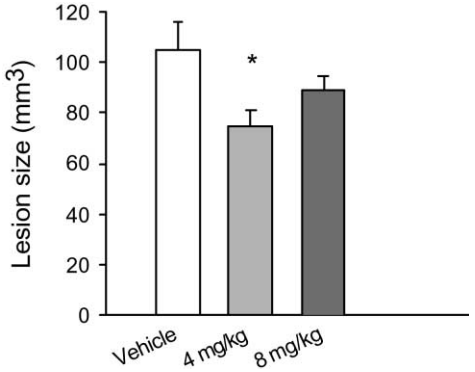


Figure 7. Rat TBI, volumes of cortical lesions: **RPR117824** was administered at 3×4 ($n = 13$) and 3×8 mg/kg ($n = 13$) at 15 min (iv) 6 and 24 h (sc) post TBI. Vehicle was administered following the same protocol ($n = 11$). One week post-TBI rats were killed and brains were removed. Areas of cerebral lesion were measured after staining of coronal cryostat brain sections with hematoxylin/eosin.

Experimental

In vitro assay

[^{3}H] AMPA binding. Radiolabeled AMPA binding assays in rat cerebral cortex membranes were performed as previously described[18] with the following modifications. Briefly, rats were decapitated and their cerebral cortices were removed on ice and immediately frozen at −80 °C for at least 1 h. The tissue was rapidly thawed,

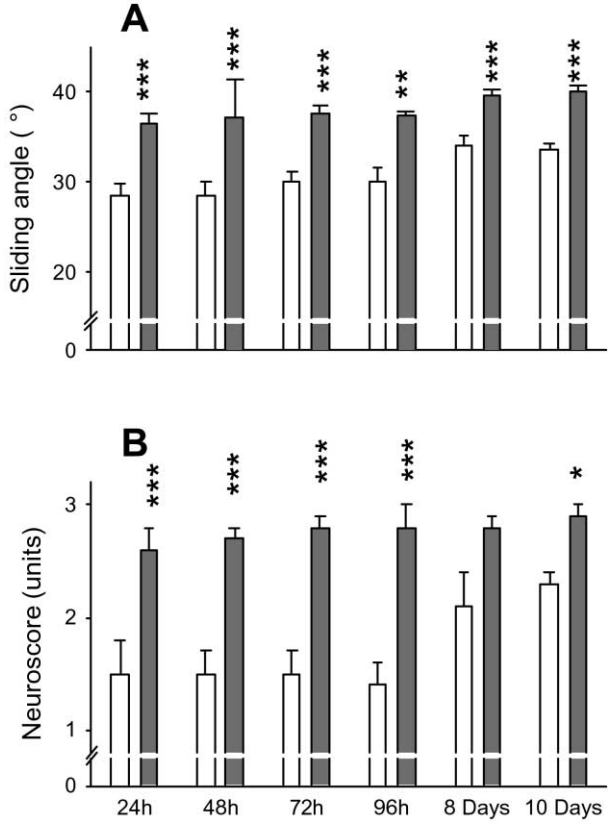


Figure 8. Rat SCI, behaviour of animals in 10 days following a spinal compression injury: (A) Slide angle of the rat placed on an inclined plane where the gradient was gradually increased. (B) Neuroscore carried out on inclined plane at 25°. 0 = unconscious, 1 = no movement in hind legs, 2 = some movement at all joints, 3 = walking and weight bearing, 4 = normal. Treated group (dark gray bars, $n = 13$). Control group (open bars, $n = 11$). Statistics, Students t-test.

homogenized with a Polytron© in 20 volumes of cold (4 °C) sucrose (0.32M) and centrifuged at 1000*g* for 20 min. The supernatant was re-centrifuged at 17,500*g* for 20 min at 4 °C. The resulting pellet was suspended in 50 volumes of ice-cold distilled water, incubated at 37 °C for 30 min and centrifuged at 32,000*g* for 20 min at 4 C. This procedure was repeated in order to remove any endogenous glutamate. The biological material thus obtained, was suspended in 50 volumes of HEPES buffer (10 mM pH 7.5) and centrifuged at 32,000*g* for 20 min at 4 °C. The pellet was finally re-suspended in 30 volumes of ice-cold HEPES buffer and was frozen at −80 °C until use. On the day of the binding assays, the membranes were thawed and centrifuged at 32,000*g* for 20 min at 4 °C. This procedure was repeated and the final pellet was suspended in a pH 7.5-buffer containing 10 mM KH_2PO_4 and 100 mM KSCN at a concentration of 0.2 mg protein/mL. Membranes were then incubated for 30 min at 4 °C with [^{3}H]AMPA (10 nM) and the compound under study or 1 mM L-glutamate for determination of the non-specific binding. The binding interaction was terminated by filtration through glass fiber filters (Printed filtermat A) for Betaplate TM scintillation counter using a Skatron micro cell harvester. The filters were immediately rinsed with 5 mL of cold buffer. The radioactivity remaining on the filters was measured by liquid scintillometry. Protein levels were measured by the method of Bradford (Bio-Rad Protein Assay).

Functional assay

Antagonist activity at heterologously expressed AMPA receptors. Oocytes were removed from ovarian lobes of anaesthetised *Xenopus laevis* (150–300 g) and defolliculated for 1 h with collagenase (2 mg mL^{-1}) in modified Barth's (MBS) medium containing NaCl (88 mM), KCl (1 mM), $MgSO_4$ (0.82 mM), $NaHCO_3$ (2.4 mM), $Ca(NO_3)_2$ (0.33 mM), $CaCl_2$ (0.41 mM), HEPES (10 mM). Oocytes were then injected with 50 mL poly(A)+ mRNAs extracted from 10- to 12-day-old rat cerebral cortex. Injected oocytes were maintained for at least 48 h at 19 °C in MBS medium supplemented with streptomycin (10 mg/mL) and penicillin (100 units/mL) before being used for voltage-clamp experiments. On the day of study, oocytes were mounted individually in a small recording chamber (300 μL) and superfused with a Ringer medium containing NaCl (90 mM), KCl (1 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1 mM), HEPES (5 mM) at a flow rate of 6–10 mL/min. Current responses were recorded with 3 M KCl (0.8–2.5 MΩ)-filled glass microelectrodes at a holding potential of −60 mV with a two-electrode voltage-clamp amplifier. **RPR117824** was prepared as a 10–2 M stock solution in H_2O diluted to the desired concentration with superfusion medium. Concentration–response curves to kainate were constructed in the absence and in the presence of increasing concentrations of **RPR117824**. The amplitudes of the evoked responses were expressed as a percentage of the maximal pre-drug baseline control responses. EC_{50} values were determined by a non-linear least square regression procedure according to a sigmoidal equation.

Inhibitory activity at a native glutamatergic synapse. Transverse hippocampal slices (approximately 0.5 mm thick) were prepared from male Sprague–Dawley rats weighing 150–200 g and mounted in a submersion-type recording chamber under continuous superfusion (flow rate = 2.5–3 mL/min) of gassed (95% O_2/5% CO_2) and thermostated (32 °C) artificial cerebrospinal fluid (ACSF) containing (in mM):NaCl 124, KCl 5, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 26, KH_2PO_4 1.25 and glucose 10. Stimulation and recording electrodes were positioned in the CA1 stratum radiatum and field excitatory post-synaptic potentials (EPSP) evoked by constant voltage stimulations delivered every 30 s at approximately 80% of maximal synaptic strength were recorded extracellularly using appropriate waveform processing and data analysis software. Field EPSP amplitude was monitored every 30 s for up to 90 min post treatment. Treatments were applied via the perfusion bath by replacing ACSF containing a defined concentration of drug for normal ACSF during 20 min. Raw data were normalized with respect to a 30-min baseline recording period preceding each treatment. The effects of **RPR117824** were compared with those of **YM90K**. Both drugs were dissolved in concentrated form in distilled water (with 1 N NaOH in the case of **RPR117824**) and diluted to the desired concentration in ACSF.

In vivo assays

Experiments were carried out on male swiss white mice (CD1, Charles River, France;) or male Sprague–Dawley rats (Charles River, France) unless otherwise stated. Control groups receiving vehicle alone were incorporated in each experiment. Compounds were administered iv in saline or by other parenteral routes in distilled water + 1% polysorbate (tween 80) at a volume of 25 mL/kg body weight for mice and 5 mL/kg body weight for rats. The ED_{50} value was calculated as the dose of compound which protected 50% of animals.[25]

Supramaximal electroshock seizures in mice. Groups of 5–10 mice (19–23 g body weight) received **RPR117824** by various routes of administration. After an appropriate lapse of time in their home cage the animals were taken by the scruff of the neck and an electric shock (75 mA, 0.3 s, 50 pulses/s) was applied immediately by ocular electrodes. Each animal was then held for 30 s, or until a tonic convulsion occurred. This could be recognized by the gathering of the hind feet up under the belly, followed by their full extension behind the body.

Pentylenetetrazole (PTZ) induces seizures in mice. Pentylenetetrazole (150 mg/kg sc), dissolved in saline was given to groups of 10 male mice (18–35 g bw) 30 min after the ip administration of drug according to the method of Everett et al.[19] Animals were then housed individually in boxes divided into 15 compartments (13 × 13 cm) and observed for occurrence of seizures during 30 min after pentylenetetrazole administration.

Bicuculline-induced seizures in mice. Bicuculline (6 mg/kg ip), dissolved in saline (25 mL/kg) was given to groups of 10 male mice (18–25 g bw) 30 min after the

sub-cutaneous administration of drug, according to the method of Curtis et al.[20] Animals were then housed individually in boxes divided into 15 compartments (13 × 13 cm) and observed for occurrence of seizures for 15 min after bicuculline administration.

Isoniazide-induced seizures in mice. Isoniazide (200 mg/kg ip), dissolved in saline (25 mL/kg) was given to groups of 5–10 male mice (18–35 g bw) at the same time of the sub-cutaneous administration of drug, according to a method adapted from Costa et al.[21]

Animals were then housed individually in boxes divided into 15 compartments (13 × 13 cm) and observed for occurrence of seizures for 150 min after isoniazide administration.

Strychnine-induced seizures. Stychnine nitrate (2.5 mg/kg ip), dissolved in saline (25 mL/kg) was given to groups of 10 male mice (18–35 g bw) 30 min after the sc administration of drug according to the method described by Lehmann et al.[22] Animals were then housed individually in boxes divided into 15 compartments (13 × 13 cm) and observed for occurrence of seizures for 15 min after strychnine administration and lethality was scored 24 h later.

4-Aminopyridine-induced convulsions in mice. 4-Aminopyridine (15 mg/kg ip), dissolved in saline (25 mL/kg) was given to groups of 10–20 male mice (18–35 g bw) 30 min after the sub-cutaneous administration of drug, according to a method adapted of Mihaly et al.[23] Animals were then housed individually in boxes and observed for occurrence of seizures for 30 min after 4-AP administration.

Harmaline-induced tremors in rats. Harmaline (30 mg/kg ip), dissolved in saline (5 mL/kg) was given to groups of five male rats weighing 130–200 g, 30 min after the sc administration of drug according to the method described by Costa et al.[22] Animals were then housed individually in boxes and observed for occurrence of seizures for 40 min after harmaline administration.

Bilateral carotid occlusion (BCO) in gerbils. Male Mongolian gerbils, *Meriones unguiculatus* (Janvier, France, bw 55–75 g) received **RPR117824** in physiological saline by subcutaneous route at various times before or after 5 min of global cerebral ischemia. Ischemia was provoked under halothane anesthesia, by the occlusion of the two common carotid arteries with aneurism clips. At the end of the ischemic episode, the arteries were unclipped, the wound sutured, and the animals returned to their home cage. Two treatment protocols were examined:

1. dose–response curve with four treatments at 0.5, 4.5, 24 and 28 h post ischaemia (animals receiving 16 mg/kg only received the first two doses);
2. therapeutic window with administrations starting 15 min before ischemia followed by 30 min, 3 h and 6 h post (isch/sham = saline at all times; 15 min. = 4, 4, 8 and 8 mg/kg product, 30 min = 3 doses of 8 mg/kg product, 3 h = 8 and 8 mg/kg product and 6 h = 8 mg/kg product, respectively).

Animal behavior was assessed 1 week after the ischemic insult.

Open field (Fig. 5A and D). The hypermotility provoked by the ischemia was assessed in an open field of 70 × 90 cm, divided into four equal rectangles. Passage from one rectangle to another was counted over a period of 3 min following 3 min habituation to the open field.

Passive avoidance learning (Fig. 5B and E). Animals were placed in a perspex container of 22 cm^2 with a floor of electrified bars (50 V) and a safe area of 22 × 9.5 cm. Animals were placed on the safe area and given 1 min to discover the electrified floor. Time spent on the electrified floor was then recorded during the 4 min period immediately following the 1 min training period.

Hippocampal histology (Fig. 5C and F). Animals were decapitated 15 days post-ischemia, brains removed and frozen in isopentane (−20 °C) Hippocampal histology was examined after cresyl violet staining of 20 μm cryostat slices. The length of undamaged pyramidal cells from the horn of the CA_3 zone to the damaged area of CA_1 pyramidal cells, which includes the CA_2 zone of the hippocampus, was measured (arbitrary units, measured with graticule under microscope at 125 × magnification) on three sequential slides taken at between 3 and 4.5 mm post bregma.[26]

Middle cerebral artery occlusion (MCAO) in rats. Male Fischer rats (Charles River France) weighing about 250 g were used. Rats were anæsthetized with halothane in a nitrous oxide–oxygen mixture (2:1). Permanent occlusion of the right middle cerebral artery (pMCAO) was performed by a subtemporal approach, essentially as described by Tamura et al.[24] Following surgery, the wound was sutured, and the animals were returned to their home cages in a room warmed at 26–28 °C. **RPR117824** was administered intravenously (iv) at 8 and 16 mg/kg at 10 or 30 min and then subcutaneously (sc) 2 h or 2 h 30 min after ischemia onset. Control rats received the vehicle (sterile H_2O) according to the same protocol. Two days after surgery, rats were killed and their brains removed and frozen. Coronal cryostat sections were stained with cresyl violet at 0.5%. Areas of infarction were measured with an image analyzer (Leica Q500) and corrected to compensate for the edema.

Traumatic brain injury (TBI) in rats. Male Sprague–Dawley rats (250–300 g bw) were anesthetized with chloral hydrate (300 mg/kg ip) or halothane N_2O/O_2 70/30 (1.5%) and placed in a stereotaxic frame. The scalp was incised, and a hole was performed with a dental drill at the level of the right parietal cortex (coordinates: 3.5 mm anterior to 6 mm above the interaural line). A 3-mm inner diameter polyethylene tube was placed over the dura, fixed securely into the craniotomy with dental cement and connected to a solenoid valve. The dura was kept intact. The opposite end of

the valve was connected to an HPLC pump. The system was filled with sterile water, and once the pump had reached the pre-determined pressure (3.8–4 bar), fluid percussion of moderate severity (1.6–1.8 bar) was induced by a brief opening (20 ms) of the valve. Following brain percussion, the tubing was removed, the scalp sutured, and the animals were returned to their home cage in a room warmed at 26–28 °C. **RPR117824** was administered 15 min (iv), 6 h (sc) and 24 h (sc) post-TBI at 4 and 8 mg/kg. Histological measure of the cortical lesions using the hematoxylin/eosin staining was performed 1 week post-TBI with an image analyzer (Leica Q500).

Spinal cord injury (SCI) in rats. Male Sprague–Dawley rats (Iffa Credo, France, 335–360 g bw) were anesthetized with sodium pentobarbital (60 mg/kg ip), opened dorsally and a Fogarty catheter (French 2) inserted at level L1-L2. The catheter was inflated with a Hartley balloon inflation syringe and held at 1.5 bar for 2 min. It was then removed and the wound closed. **RPR117824** in physiological saline was administered intravenously 30 min post trauma at 4 mg/kg. Animals were then retreated 4 h after injury at 4 mg/kg sc. Subsequent doses at 8 mg/kg sc were given 24, 48 and 72 h post trauma. Control traumatized animals received saline at the same intervals, as did sham-operated rats. The behavior of the animals was assessed on an inclined plane prior to each injection. An evaluation of the angle of slide on the plane was made and a neurological score was assessed as follows: 0 = unconscious, 1 = no movement in hind legs, 2 = some movement at all joints, 3 = walking and weight bearing, 4 = normal.

Statistics. Statistical comparisons were made using the software Instat 3.0 (GraphPad Software Inc., San Diego, CA, USA). Asterisks denote significant differences as follows: * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$.

Chemistry

General. 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. All ^{1}H NMR spectra were recorded at 250 or 300 MHz using Bruker AC spectrometers. Chemical shifts are reported in δ ppm with DMSO as internal standard (δ 2.5 ppm) while coupling constants are given in Hz. ^{1}H are identified according to numbers provided in Scheme 1. IR spectra (KBr dispersions) were recorded between 4000 and 400 cm^{-1} using a 60SXR Nicolet FT-IR spectrophotometer at a 2 cm^{-1} resolution. Mass spectra were obtained either on a VG Autospec (LSIMS Cs gun at 35 kV; in glycerol/thioglycerol as a matrix) or on a Finnigan SSQ 7000 (CI, NH_3). Elemental analyses (% C, H, N, O) were determined using a Fisons 1108 Micro-analyzer. Sodium contain was measured using a Perkin-Elmer 4100 atomic absorption spectrometer while % in water was obtained using a Karl Fischer titrimeter.

(2-Bromo-1-oxo-indan-4-yl)-acetic acid (7). To a solution of (1-oxo-indan-4-yl) acetic acid[16a] **6** (38 g, 0.2 mol) and 47% hydrobromic acid (4 mL) in glacial acetic acid (800 mL) was added, dropwise, bromine (11 mL, 0.21 mol) maintaining a temperature around 15 °C. The reaction mixture was stirred for 2 h at room temperature. Then, it was poured into ice-water mixture (800 mL) and extracted with methylene chloride (2 × 800 mL). The organic phase was shaken with water, dried over $MgSO_4$, filtered, and evaporated to dryness. The crude material was crystallised from di-isopropyl ether to give **7** (45.4 g, 84%) as a off-white solid (mp 113 °C). R_f 0.36 in CH_2Cl_2–MeOH mixture (98/2). MS (CI, NH_3): m/z 286 and 288 (MNH_4^+); IR (KBr) cm^{-1}: 1730, 1715, 1415. NMR (300MHz, DMSO) δ 3.3 (1H, dd, J = 18 and 1.5 Hz, H_3), 3.75 (2H, s, CH_2COOH), 3.85 (1H, dd, J = 18 and 7.5 Hz, H_3), 5.05 (1H, dd, J = 7.5 and 1.5 Hz, H_2), 7.5 (1H, t, J = 8.5 Hz, H_6), 7.70 (2H, br.d, J = 8.5 Hz, H_5 and H_7), 12.6 (1H, very br.s, COOH). A strong NOE was observed between H_3 at δ 3.3 and the CH_2COOH at δ 3.75.

(2-Bromo-1-oxo-indan-4-yl)-ethyl acetate (8). To a stirred solution of **7** (38 g, 0.141 mol) in methylene chloride (500 mL) and ethanol (100 mL) was added dropwise a solution of oxalyl chloride (19.7 g, 0.155 mol) in methylene chloride (100 mL).The addition rate (30 min) was adapted to maintain a temperature around 30 °C. The reaction mixture was stirred at room temperature for 2 h, and concentrated to dryness under vacuum. The residue was extracted with methylene chloride (500 mL), washed with water (2 × 230 mL), dried over $MgSO_4$, filtered and concentrated to dryness under vacuum. The residue was then purified by flash chromatography on silica (5% ethyl acetate in methylene chloride) and crystallised from diisopropyl ether to give **8** (17 g, 53%) as a yellow oil. R_f 0.52, CH_2Cl_2. MS (CI, NH_3): m/z 314 and 316 (MNH_4^+); IR (KBr) cm^{-1}: 2980, 1730, 1590, 1275, 1212, 1030, 670. NMR (300 MHz, DMSO) δ 1.2 (3H, t, J = 7 Hz, CH_3), 3.3 (1H, dd, J = 18 and 1.5 Hz, H_3), 3.85 (3H, m, H_3 and CH_2COO), 4.15 (2H, q, J = 7 Hz, CH_2O), 5.05 (1H, dd, J = 7.5 and 1.5 Hz, H_2), 7.5 (1H, t, J = 8.5 Hz, H_6), 7.7 (2H, br.d, J = 8.5 Hz, H_5 and H_7).

1-(4-Ethoxycarbonylmethyl-1-oxo-indan-2-yl)-2,4-diethoxycarbonyl imidazole (10). To a solution of **9**[17] (1.5 g, 5 mmol) and potassium carbonate (3.45 g, 25 mmol) in acetone (30 mL) at reflux was added, dropwise, a solution of **8** (1.7 g, 5 mmol) in acetone (10 mL). The reaction mixture was refluxed for 1 h, and then concentrated to dryness. The residue was extracted with methylene chloride (3 × 50 mL), washed with water (2 × 25 mL), dried over $MgSO_4$, filtered and concentrated to dryness under vacuum. The crude material was crystallised from diisopropyl ether to yield **10** (1 g, 82%) as a yellow solid (mp 120 °C). R_f 0.6 in CH_2Cl_2–MeOH mixture (95/5). MS (CI, NH_3): m/z 429 (MH^+); IR (KBr) cm^{-1}: 1730,1720 (sh.),1705 (sh.), 1200, 1030. NMR (250 MHz, DMSO) δ 1.15 (6H, m, 2 CH_3), 1.3 (3H, t, J = 7 Hz, CH_3), 3.35 (1H, dd, J = 18 and 6 Hz, H_3), 3.75 (1H, dd, J = 18 and 7 Hz, H_3), 3.85 (2H, s, CH_2COO), 4.1 (4H, m, 2 CH_2O), 4.25 (2H, q, J = 7 Hz, CH_2O), 5.85 (1H, dd, J = 7 and 6 Hz, H_2), 7.5 (1H, t,

$J = 8.5$ Hz, H6), 7.65 (2H, br.d, $J = 8.5$ Hz, H_5 and H_7), 8.25 (1H, s, imidazole).

Ethyl 9-ethoxycarbonylmethyl-imidazo[1,2-*a*]indeno[1,2-*e*]-pyrazin-4-one-2-carboxylate (11). A mixture of **10** (15.2 g, 40 mmol), glacial acetic acid (140 mL) and ammonium acetate (30.8 g, 0.4 mol) was refluxed for 3 h. The reaction mixture was then filtered and dried under vacuum at 60 °C. The crude solid was recrystallized from DMF to yield **11** (12 g, 77%) as a grey solid (mp > 260 °C. R_f 0.52 in CH_2Cl_2–MeOH mixture (90/10). MS (CI, NH_3): *m/z* 382 (MH^+); IR (KBr) cm^{-1}: 3120, 1730, 1675, 1295, 1150, 1025, 780. NMR (250 MHz, DMSO) δ 1.25 (3H, t, $J = 7$ Hz, CH_3), 1.35 (3H, t, $J = 7$ Hz, CH_3), 3.8 (2H, s, CH_2COO), 4 (2H, s, H_{10}), 4.15 (2H, q, $J = 7$ Hz, CH_2O), 4.35 (2H, q, $J = 7$ Hz, CH_2O), 7.2 (1H, dd, $J = 8.5$ and 1.5 Hz, H_8), 7.40 (1H, t, $J = 8.5$ Hz, H_7), 7.8 (1H, dd, $J = 8.5$ and 1.5 Hz, H_6), 8.6 (1H, s, H_1), 12.6 (1H, very br.s, NH). The CH_2COO and H_1 gave strong NOE enhancements with H_{10}.

9-Carboxylatemethyl-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one-2-carboxylate, tris sodium salt, $5H_2O$ (12). To a solution of **11** (11.4 g, 30 mmol) in water (400 mL) and dioxane (1500 mL) was added, dropwise, 1 N NaOH (120 mL, 0.12 mol). Then, the reaction was stirred under argon at room temperature for 4 h. The solid was filtered, washed successively with a solution of 7% of water in dioxane (2 × 100 mL) and finally dioxane (3 × 75 mL), and dried under vacuum at 50 °C to yield **12** (14 g, 94%) as a off-white hydroscopic solid (mp > 260 °C). MS (FAB): *m/z* 348 (MNa^+); IR (KBr) cm^{-1}: 1580, 1525, 1400, 1365, 1340, 650. NMR (300MHz, D_2O; δ $D_2O = 4.7$ ppm) δ 3.3 and 3.4 (2 × 2H, 2 s, H_{10} and CH_2COO), 6.8 (1H, br.d, $J = 8.5$ Hz, H_8), 7.1 (1H, t, $J = 8.5$ Hz, H_7), 7.35 (1H, br.d, $J = 8.5$ Hz, H_6), 7.8 (1H, s, H_1). Elemental analysis: % calcd C 39.93, H 3.77, N 8.73, Na 14.33; found C 39.64, H 3.88, N 8.64, Na 14.6. Water: % calcd 17.8; found 18.2.

9-Carbonylmethyl-imidazo[1–2*a*]indeno[1–2*e*]pyrazin-4-one-2-carboxylic acid (RPR117824). To a solution of **12** (12.4 g, 0.031 mol) in water (220 mL) was added, dropwise, a 0.3N hydrochloric acid solution (330 mL, 0.165 mol) while a temperature around 25 °C was maintained. The reaction mixture was stirred for 1 h at room temperature. The solid was then filtered and washed with water (2 × 100 mL) then methanol (70 mL) and acetone (2 × 70 mL), dried under vacuum at 60 °C to lead **RPR117824** (7.68 g, 94%) as a white solid (mp > 260 °C). MS (CI, NH_3): *m/z* 343 (MNH_3^+); IR (KBr) cm^{-1}: 2940, 1675, 1530, 1280, 775. NMR (250 MHz, DMSO) δ 3.75 (2H, s, CH_2COO), 4 (2H, s, H_{10}), 7.25 (1H, br.d, $J = 8.5$ Hz, H_8), 7.4 (1H, t, $J = 8.5$ Hz, H_7), 7.8 (1H, br.d, $J = 8.5$ Hz, H_6), 8.5 (1H, s, H_1), 12.4 (1H, s, NH), 12.75 (2H, very br.d, COOH). Elemental analysis: % calcd C 59.08, H 3.41, N 12.92, O 24.59; found C 59.14, H 3.57, N 12.94, O 24.45.

Acknowledgements

We gratefully acknowledge the technical assistance of F. Bordier, D. Briet, J. Lafforgues, M. Roux.

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