Synthesis and Structure-Activity Relationships of 4,10-Dihydro-4-oxo-*4H*-Imidazo[1,2-*a*]Indeno[1,2-*e*]Pyrazine Derivatives: Highly Potent and Selective AMPA Receptor Antagonists with *In Vivo* Activity

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Abstract: The excitatory neurotransmitter glutamate interacts with ionotropic and metabotropic receptors that mediate a variety of normal signalling processes in the brain. However, excessive stimulation of these receptors appears to be involved in neurodegenerative processes, at least in animal models. Ionotropic glutamate receptors can be divided into NMDA and non-NMDA (AMPA and KA) subtypes on the basis of t heir preferential affinities for the synthetic excitatory aminoacids *N*-methyl-D-aspartic acid (NMDA) or 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid (AMPA), respectively. Although most of the early evidence favoured a role for NMDA receptors in the excitotoxic processes, it has been recognised that AMPA receptors may also be significantly involved in neuronal death. As a consequence, the synthesis of specific AMPA antagonists has raised much interest as source of potential drugs for epilepsy and acute and chronic neurodegenerative diseases. The discovery of **RPR117824**, a potent and selective AMPA receptors antagonist endowed with anticonvulsant and neuroprotective properties, induced growing interest on dihydro-4-oxo-*4H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine series. This review covers the main chemical course leading to the most promising compounds as well as the pharmacological properties of this original class of AMPA receptor antagonists.

†*Dedicated to the memory of Dr. Marc Vuilhorgne*

INTRODUCTION

Over the last 20 years, L-Glutamate has been recognized as one of the most potent neurotransmitter mediating synaptic excitation in the mammalian central nervous system. The excessive release of glutamate is thought to play an important role in a variety of neurological disorders such as cerebral ischemia following cardiac arrest, thromboembolic or haemorrhagic stroke, hypoglycaemia, epilepsy, cerebral or spinal trauma. Glutamate excitotoxicity has also been implicated in chronic disorders such as Parkinson's Huntington's and Alzheimer's Diseases. Consequently, excitatory amino acid antagonists may have an important therapeutic potential in the treatment of these diseases [1]. Glutamate interacts with at least three type of receptors: (*i*) NMDA (N-methyl-D-aspartic acid) receptors;

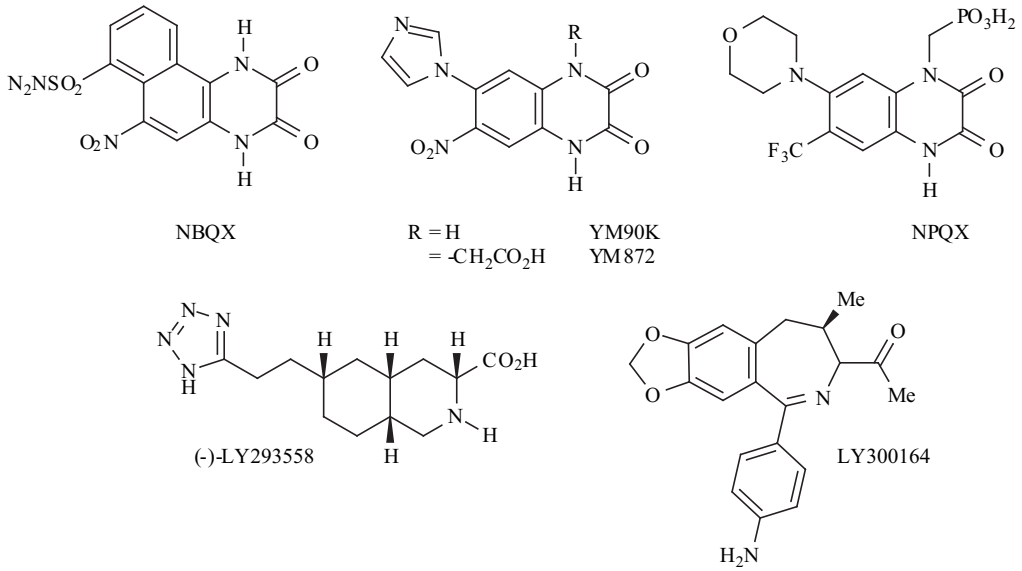

Fig. (1). Chemical structure of NBQX, YM90K, YM872, NPQX, (-)-LY293558 and LY300164.

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1389-5575/04 $45.00+.00

(*ii*) AMPA [2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid] / KA (kainic acid) receptors and (*iii*) metabotropic receptors. The former two types are ionotropic, whereas the latter are linked to a G-protein second messenger [2]. Blockade of AMPA/KA receptors has been suggested to prevent ischemic insult in experimental models. AMPA receptors are composed of four subunits (GluR1 to GluR4) that are widely, but differentially, expressed throughout the CNS and form functional receptors as heterotetrameric subunit assemblies [3].

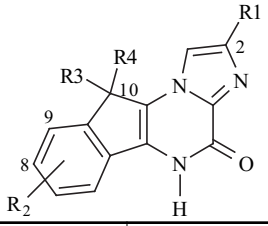
Heretofore described antagonists at AMPA receptors belong to the following chemical families [4]: i) quinoxalines, ii) heterocyclic-fused quinoxalinones, iii) isatinoximes, iv) quinazolines, v) quinolones, vi) decahydroisoquinolines and vii) dihydrophthalazines. Representative examples of these classes are **NBQX** [5], **YM90K** [6], **YM872** [7], **NPQX** [8], **(-)-LY 293558** [9] and **LY-300164** [10]. To date, **YM-872** and **LY-300164** (talampanel) have been evaluated in clinical trials Phase I/II for cerebrovascular ischemia (Figure **1**) [11].

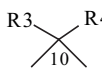
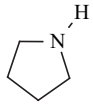
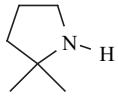
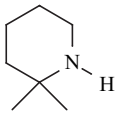
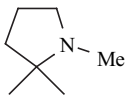
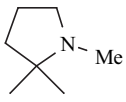
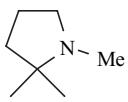
As part of our program directed towards the development of new excitatory amino acid antagonists based on the recently identified anticonvulsant and neuroprotective lead compound imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one (**1**) [12], we prepared compounds with improved *in vitro* and *in vivo* activities. This paper is aimed at providing a summary of the chemistry and structure-activity relationships of original dihydro-4-oxo-*4H*-imidazo[1,2-*a*]indeno[1,2-*e*]-pyrazine derivatives [13].

CHEMISTRY

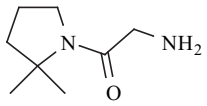
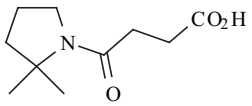
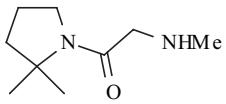
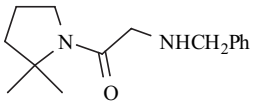
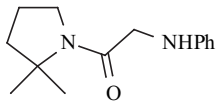
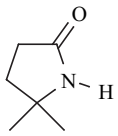
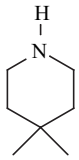
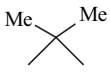
As shown in the Scheme 1, our investigations into the design of novel potent and selective compounds belonging to this pharmacological class started with the initial discovery of the anticonvulsant and neuroprotective properties of imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one **1** [12] showing moderate and anticonvulsant properties in mice and rats, and neuroprotective properties in models of global cerebral ischemia in the gerbil as well as focal cerebral ischemia and neurotrauma in the rat (Table 1).

Table 1. ***In Vitro*** **and** ***in Vivo*** **Activities of 10-Substituted-4-Oxo-Imidazo[1,2-*a*]Indeno[1,2-*e*]Pyrazine Derivatives**

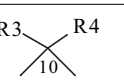
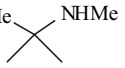
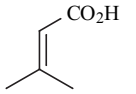
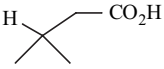
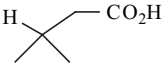
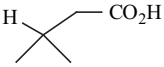
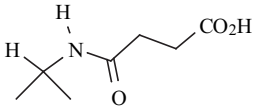
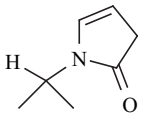
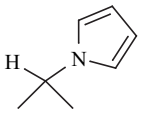
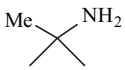
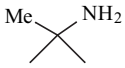
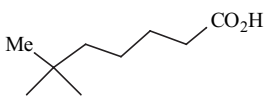


Compd.	R1	R2		Receptor affinity[a] IC_{50} (nM)		Anticonvulsant activiy ED_{50} (mg/kg)
				AMPA	NMDA	MES[b] *i.p.*[c] (*i.v.*[d]) DBA/2 *i.p.*[e]
1	H	H	$-CH_2-$	760	3000	62
8	H	H		200	640	-
9a	H	H		250	280	80
9b	H	H		390	1180	45
9c	H	H		200	420	31 (10)
(-)-9c	H	H		4900	1160	80
(+)-9c	H	H		86	172	17 (7)

(Table 1). contd.....

Compd.	R1	R2	R3, R4, 10	Receptor affinity[a] IC_{50} (nM)		Anticonvulsant activiy ED_{50} (mg/kg)
				AMPA	NMDA	MES[b] *i.p.*[c] (*i.v.*[d]) DBA/2 *i.p.*[e]
9d	H	H		620	530	54
9e	H	H		4300	470	-
9f	H	H		10,000	1300	-
9g	H	H		830	2800	70
9h	H	H		360	10,000	10
9i	H	H		4000	1700	-
9j	H	H		10,000	1700	-
9k	H	H		7100	130	-
10	H	H		1500	1700	-
11	H	H		380	2000	-
12a	H	H		2300	600	-
12b	H	H		1600	1400	-
12c	H	H		4400	1000	-

(Table 1). contd.....

Compd.	R1	R2		Receptor affinity[a] IC_{50} (nM)		Anticonvulsant activiy ED_{50} (mg/kg)
				AMPA	NMDA	MES[b] *i.p.*[c] (*i.v.*[d]) DBA/2 *i.p.*[e]
12d	H	H		3400	2700	-
(E)-26	H	8-NHCONHMe		19	100,000	11 (5.6) 1.6
28	H	8-NHCONHMe		8	14,000	1.8 (1) 0.6
(-)-28	H	8-NHCONHMe		39	10,000	7 4.4
(+)-28	H	8-NHCONHMe		4	10,000	1 0.9
30	$-CO_2H$	H		93	158	19 (7)
31	$-CO_2H$	H		35	230	2.5 (1.7)
32a	$-CO_2H$	H		23	184	20 (7.7)
32b	$-CO_2H$	H		55	32	26 (10)
35	H	8-NHCONHMe		51	14,000	5.7 4.7
(-)-35	H	8-NHCONHMe		42	>100,000	80
(+)35	H	8-NHCONHMe		10	32000	10 (2.5) 1.8
37	$-CO_2H$	8-NHCONHMe	$-CH_2-$	9	4330	0.7 0.5
39	$-CO_2H$	H		242	134	10 (9.5)

a: IC_{50} values are mean of at least 3 determinations, each with at least 3 concentrations of tested compound in triplicate. **b**: ED_{50} values (in mg/kg) are defined as the dose which protected 50% of the animals from a tonic convulsion (6 male CD1 mice/dose of compound, with at least 3 doses compared to a group receiving vehicle alone) **c** : in mouse, pre-treatment time: 30 min, vehicle: 1% Tween-80 in water. **d** : in mouse, pre-treatment time: 5 min, vehicle: saline
e : in mouse, pre-treatment time: 30 min, vehicle: saline

Synthesis of Imidazo[1,2-a]Indeno[1,2-e]Pyrazin-4-One (1)

The synthesis of (**1**) was first carried out *via* a demethylation reaction (Pathway 1) of the crude pyrazinium salt (**4**) using an excess of imidazole (30 eq.) in neat phase at high temperature (160°C) giving (**1**) with 33% yield. Pyrazinium salt (**4**) was prepared by condensation of the imidazocarboxamide (**3**) with the 2-bromoindanone (**2**) in neat phase at 180°C with a 94% yield.

In order to obtain (**1**) with a better overall yield a different synthetic approach was developed. Thus, we prepared (**1**) with a higher overall yield (70%) in a three-step synthesis following (Pathway 2). The condensation of the ethyl-(2-imidazolyl)carboxylate sodium salt (**5**) with (**2**) in DMF gave (**6**) with a 70% yield. Amminolysis of the ester group of (**6**) was then performed by reaction with concentrated ammonia solution in methanol to give (**7**). Finally, pyrazinone ring formation was performed by heating (**7**) in a concentrated aqueous solution of hydrochloric acid, to give pure (**1**).

Synthesis of 10-Spiro-Imidazo[1,2-a]Indeno[1,2-e]Pyrazin-4-Ones (8-12)

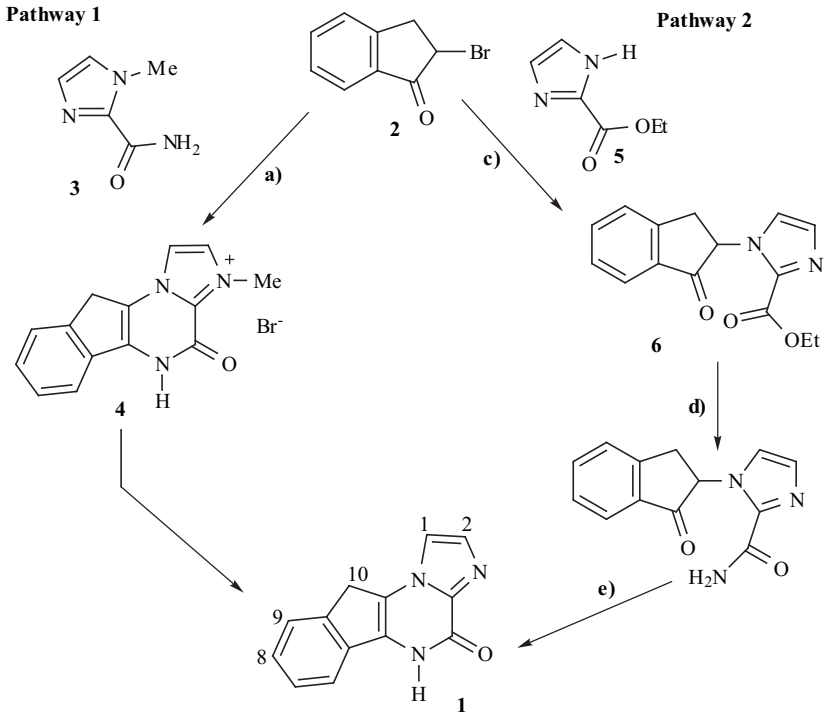
Having (**1**) in hand, important efforts led to the preparation of various original fused imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one derivatives in order to improve the *in vitro* and *in vivo* activities of this scaffold. One of the first step in our investigations was to determine the influence of substitution in position 10. The first targeted compounds were the preparation of 10-spiro-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one derivatives (**8-12**). These compounds were synthesized from (**1**) according to the sequences outlined in Scheme 2. One of these new compounds, the spiro-imidazo-indeno-pyrazin-4-one ((**+**)**-9c**) exhibited affinities at both the AMPA receptor and the glycine site of the NMDA receptor (Table 1) and displayed good *in vivo* activities in anticonvulsant tests (Table 1).

Synthesis of (8)

Compound (**1**) reacted with *tert*-butoxy-bis(dimethyl-amino)methane followed by hydrolysis using HCl and then reduction by $NaBH_4$ to give (**15**) with 10% overall yield. Then, this compound was dehydrated using NaOH to afford (**16**) with 75% yield. As the key step, regioselective [3+2] cycloaddition reaction of (**16**) with the non-stabilized azomethine ylide (**17**) (obtained *in situ* by action of TFA with *N*-benzyl,*N*-*n*-butoxymethyltrimethyl-silylmethylamine) followed by *N*-deprotection reaction (33% yield) gave pure (**8**) with 41% yield.

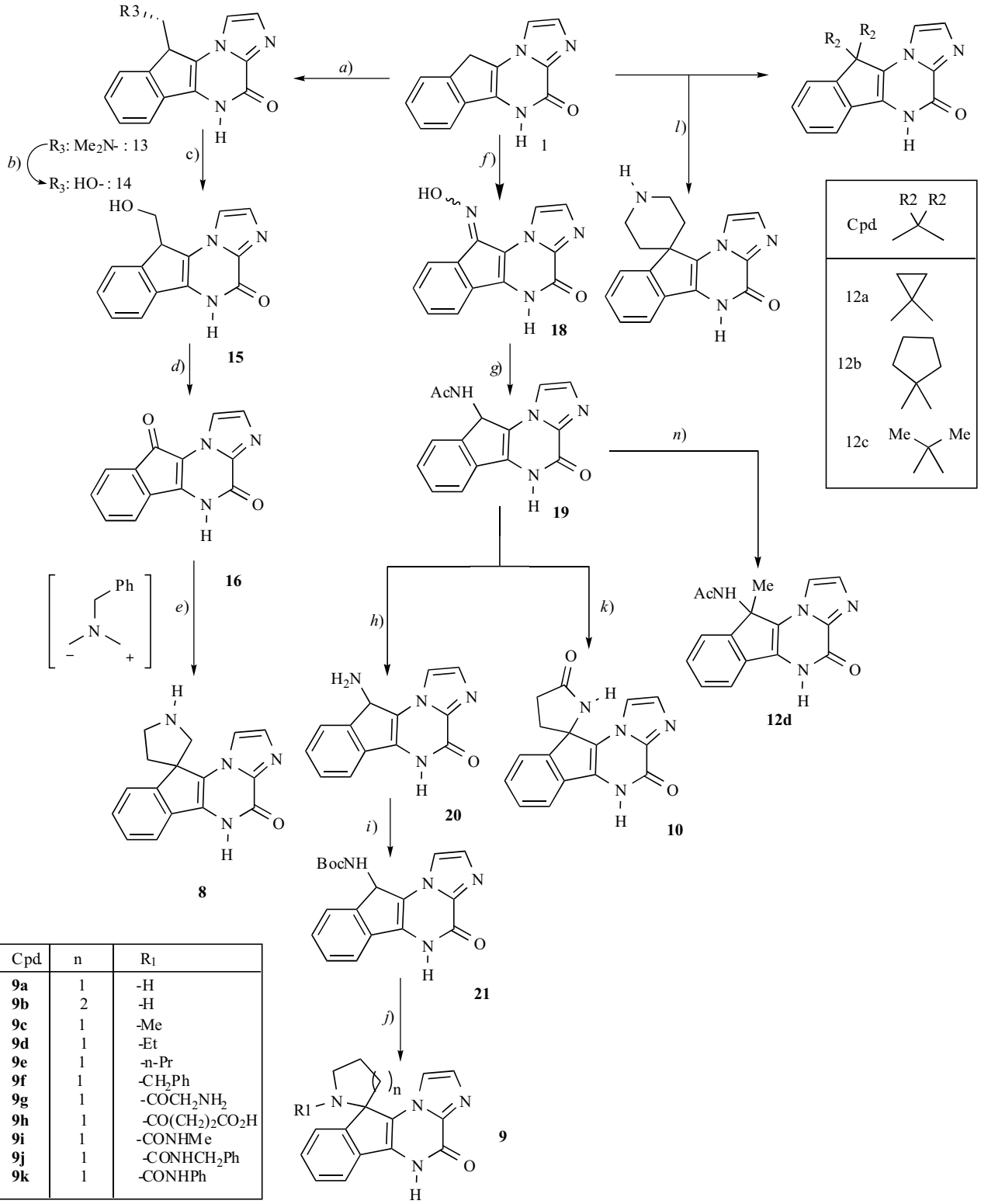
Synthesis of (9)

The 10-*N*-substituted derivatives (**19**) were prepared by treatment of (**1**) with *i*$AmNO_2$ in the presence of NaH followed by the action of Zn (powder) in acetic acid with 6% overall yield. Then, *N*-acetyl deprotection and finally *N*-Boc formation afforded (**21**) with 11% overall yield. Reaction of (**21**) with 1-chloro-3-bromopropane and 4-chloro-bromobutane as electrophiles in the presence of NaH, followed by *N*-deprotected using TFA afforded (**9a**) with a 26% and (**9b**) with a 60% overall yield, respectively. Compound (**9a**) was further converted into various *N*-substituted analogs either *via* : i) reductive alkylation with formaldehyde and formic acid ((**9c**), 54% yield), ii) *N*-ethylation ((**9d**), 26% yield), iii) *N*-propylation ((**9e**), 47% yield) by action of acetic acid and propionic acid in the presence of $NaBH_4$ and finally iv) *N*-Benzylation ((**9f**), 55% yield) under standard experimental reaction conditions with benzylbromide in the presence of KOH as base. Compound



Scheme 1. a) 180°C, 94% b) imidazole, 160°C, 33% c) *i.* NaH, DMF, rt *ii.* DMF, rt, 1h, 70% d) 2.5N NH_3-methanol, rt, 100% e) *i.* 10N aq. HCl, rt then reflux 10 min. *ii.* 1N aq. HCl, reflux until complete dissolved, 100%.

(**9g**) (10% yield) was synthesized using the condensation of *N*-phthaloylglycine chloride followed by the action of hydrazine, whereas, direct condensation of (**9a**) with succinic anhydride in acetic acid medium gave (**9h**) (29% yield). The synthesis of the ureas (**9i-k**) was carried out by the condensation with the corresponding isocyanates with good yields (>77%).



Cpd.	n	R_1
9a	1	-H
9b	2	-H
9c	1	-Me
9d	1	-Et
9e	1	-n-Pr
9f	1	$-CH_2Ph$
9g	1	$-COCH_2NH_2$
9h	1	$-CO(CH_2)_2CO_2H$
9i	1	-CONHMe
9j	1	$-CONHCH_2Ph$
9k	1	-CONHPh

Scheme 2. a) *tert*-$BuOCH(NMe)_2$, rt b) 5N HCl, rt c) $NaBH_4$, MeOH, rt d) 1N NaOH, MeOH/DMSO, rt then 1N HCl e) *n*$BuOCH_2N(CH_2Ph)CH_2SiMe_3$, cat. TFA, DMF, rt then 60°C f) NaH, *i*$AmNO_2$, DMSO, rt g) Zn, AcOH, 80°C h) 2N HCl, reflux i) *di-tert*-butyl dicarbonate, Et_3N, DMF, 25°C j) NaH, rt, n = 1: $Br(CH_2)_3Cl$; n = 2: $Br(CH_2)_4Br$ k) $Br(CH_2)_2CO_2Me$, NaH, DMSO, rt l) *p*-$MePhSO_2N[(CH_2)_2Cl]_2$, NaH, DMSO, rt m) TBAB, NaOH, DMSO, rt, $Br(CH_2)_2Br$, $Br(CH_2)_4Br$ and MeI n) *i)* NaH, DMSO, rt, MeI *ii)* 2N HCl, reflux *iii)* di-tert-butyl dicarbonate, DMF, 60°C *iv)* NaH, MeI, DMF *v)* TFA, rt then HCl, MeOH.

The good AMPA and glycine-binding site affinities of (**9c**) prompted us to examine the enantiomers ((**+**)**-9c**) and ((**-**)**-9c**). These two enantiomers (enantomeric homogeneity of both enantiomers: >99%) were prepared in optically pure form by preparative HPLC using a column packed with a chiral stationary phase (Chiracel OC phase) and ethanol as mobile phase, ((**+**)**-9c**) **:** α_D^{20} = +32.4 (AcOH, c = 0.5) ; ((**-**)**-9c**) **:** α_D^{20} = -32.0 (AcOH, c = 0.5).

Synthesis of (10) and (11)

The spiro-derivative (**10**) was obtained with a poor 4% yield by the direct condensation of (**19**) with either methyl 3-bromopropionate in the presence of NaH, whereas (**11**) was obtained by the condensation of (**1**) with *N*,*N*-bis-(2-chloroethyl)-*p*-toluenesulfonamide in the presence of NaH followed by *N*-deprotection with a 7.5% overall yield.

Synthesis of (12a-d)

The spiro-derivatives (**12a**), (**12b**) and the compound (**12c**) were prepared directly from (**1**) using catalytic phase transfer conditions in the presence of NaOH and TBAB with 12-31% yield. The compound (**12d**) was obtained in five-step synthesis from (**19**): *i)* regioselective 10-N*H* alkylation using catalytic phase transfer conditions, *ii)* 10-*N*-deacethylation reaction with HCl, *iii)* regioselective 10-N-protection using *di-tert-butyl* dicarbonate, *iv)* regioselective 10-alkylation using MeI and NaH, *v)* N-deprotection with TFA. (**12c**) was prepared with 7.5% overall yield.

Synthesis of 8-Methylureido-4-oxo-Imidazo[1,2-*a*]Indeno[1,2-*e*]Pyrazin Derivatives ((*E*)-26), (28), (35) and (37), and 4-oxo-10-Substituted-Imidazo[1,2-*a*]Indeno[1,2-*e*]Pyrazin-2-Carboxylic acid Derivatives (31), (32) and (39)

By further exploration of the bio-chemical space we synthesized 8-methylureido-imidazo-indeno-pyrazine derivatives (Scheme 3). Compounds ((***E***)**-26**) [15], ((**+**)**-28**) [15], (**31**) [16], ((**+**)**-35**) [17], (**37**) [18] and (**39**) [16] demonstrated high selective binding affinity for the AMPA receptor and very good activity *in vivo* in rodent models of convulsions (Table 1).

Synthesis of ((E)-26)

Compound ((***E***)**-26**) was prepared in a five-step synthesis from (**1**): *i)* condensation of glyoxylic acid followed by a dehydratation reaction with $ZnCl_2$-acetic anhydride affording (**23**) (15% overall yield), *ii)* regioselective nitration using potassium nitrate producing (**24**) (92% yield), *iii)* regioselective reduction of the nitro group using $SnCl_2$ in concd HCl medium (94% yield) and *iv)* condensation of methylisocyanate (35%).

Synthesis of 28

Pure compound (**28**) was prepared in a four steps from the key compound (**24**): *i)* reduction of both the nitro and ethylenic groups using conc.HCl/Fe in MeOH and *in situ* esterification of the carboxylic group leading to (**27**) (83.5% yield), *ii)* condensation of methylisocyanate followed by a preparative HPLC purification using Chiracel OD as stationary phase and eluted by a mixture of heptane/ethanol (30/70) with 0.1% of TFA giving the pure two enantiomers. Then, compounds ((**+**)**-28**) and ((**-**)**-28**) were readily saponified by action of HCl affording ((**+**)**-28**) **:** α_D^{20} = +94.2 (DMF, c = 0.5) ; ((**-**)**-28**) **:** α_D^{20} = -83.8 (DMF, c = 0.5) with ~17% and overall yield).

Synthesis of (31) and (32)

Compound (**29**) was prepared by treatment of (**22**) with *i*$AmNO_2$ in the presence of NaH with 25% yield. It was then treated with Zn powder in ammonium acetate in EtOH and ammonia (28%) at reflux followed by the action of HCl to give the 10-amino derivative (**30**) with 47% yield. Condensation of (**30**) in the presence of sodium acetate and glacial acetic acid with succinic anhydride afforded (**31**) with 66% yield, whereas its condensation (under same reaction conditions) with 2,5-dimethoxy-2,5-dihydrofurane and 2,5-dimethoxytetrahydrofurane lead to (**32a**) and (**32b**) with 18% and 41% yield, respectively.

Synthesis of (35)

The regioselective methylation of (**19**) in position 10 using methyl iodide as the electrophile and NaH as base gave (**33**) with 34.5% yield. Then, regioselective nitration using using potassium nitrate in concentrated sulfuric acid produced (**34**) with 76.5% yield. Finally, hydrogenation of the nitro group of (**34**) in presence of cat. Pd/C (10%) (70.5% yield) followed by the condensation of methylisocyanate and *N*-acetyl deprotection with HCl afforded racemic compound (**35**) with 6% overall yield.

The two enantiomers ((**+**)**-34**) and ((**-**)**-34**) were prepared in optically pure form (enantiomeric homogeneity: >90%) from the racemic compound (**35**) by preparative HPLC using Chiracel OD as stationary phase eluted by a mixture of heptane/ethanol (20/80) with 0.05% of TFA, ((**+**)**-35**) **:** α_D^{20} = +81.0 ($MeCO_2H$ c = 2); ((**-**)**-35**) **:** α_D^{20} = -90.3 ($MeCO_2H$, c = 2). (**+**)**-34**) and ((**-**)**-34**) were obtained with 49% yield and 59.6% yield, respectively.

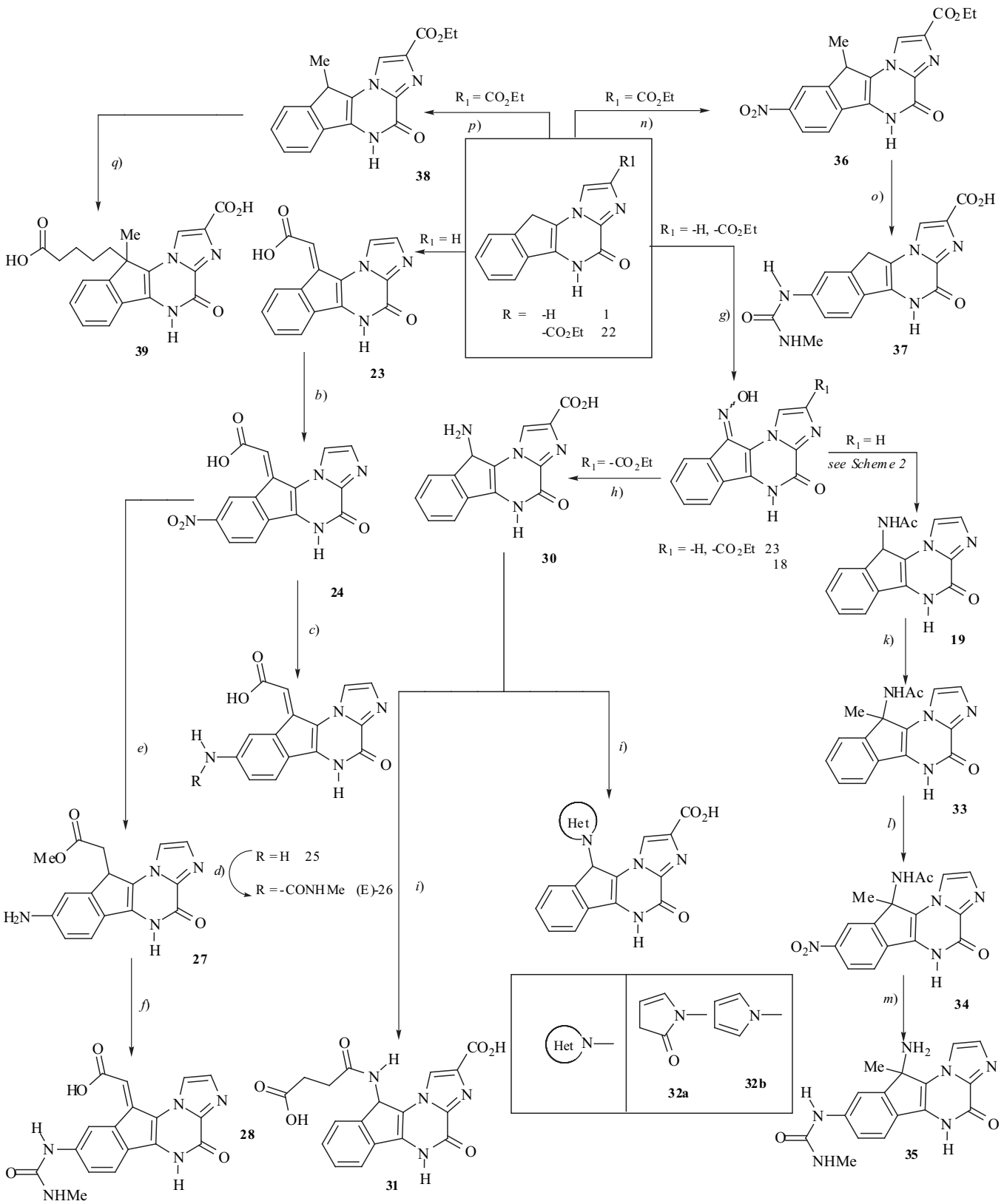
Synthesis of (37)

The 8-methylureido-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylate derivative (**37**) was obtained in a four-steps synthesis with 17% overall yield by: *i)* regioselective nitration of (**22**) using potassium nitrate in concentrated sulfuric acid, *ii)* hydrogenation of the nitro group in presence of cat. Pd/C (10%), *iii)* addition of methylisocyanate in DMF and *iv)* saponification using 1N NaOH.

Synthesis of (39)

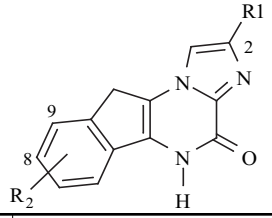
The valeric acid derivative (**39**) was prepared following a four-steps synthesis from (**22**). *i)* action of *tert*-butoxy-bis(dimethylamino)methane followed by hydrogenation of the enamine derivative intermediate in the presence of Pd/C (10%) giving (**38**) with 53% overall yield, *ii)* regioselective alkylation of position 10 with ethyl 5-bromovalerate in the presence of NaH followed by hydrolysis of the ester function (NaOH) and then action of HCl (12% overall yield).

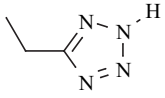
As shown in Table 1, the ureido derivatives ((**+**)**-28**), ((**+**)**-35**) and (**37**) exhibited nanomolar affinities for the AMPA receptors with good selectivities against the glycine-binding site. In addition, these compounds also displayed potent anticonvulsant properties against electrically-induced convulsions after systemic administration (Table 1).



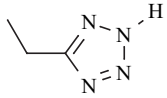
Scheme 3. a) *i)* $HOCOCO_2H$, NaH, DMF, rt then 1N HCl, rt *ii)* $ZnCl_2$, Ac_2O, reflux b) KNO_3, conc.H_2SO_4, rt c) $SnCl_2$, conc.HCl, 40°C d) MeNCO, K_2CO_3, DMF-dioxane 1-1, rt e) MeOH, conc.HCl, Fe, 65°C f) *i)* MeNCO, K_2CO_3, DMF, rt *ii)* preparative HPLC (Chiracel OD stationary phase, eluent : 3/7 heptane/ethanol with 1% of TFA) *iii)* 8N HCl, dioxane, 40°C g) $Me_2CHCH_2NO_2$, NaH, DMSO, rt h) $AcNH_4$, NH_3 (28%), Zn, EtOH, reflux then 6N HCl i) AcNa, AcOH, succinic anhydride, 50°C j) AcNa, AcOH, 2,5-dimethoxytetrahydrofurane or 2,5-dimethoxy-2,5-dihydrofurane, reflux k) NaH, DMSO, MeI, rt l) KNO_3, conc.H_2SO_4, rt m) *i)* MeNCO, K_2CO_3, DMF, rt *ii)* 6N HCl n) KNO_3, conc.H_2SO_4, rt o) *i)* H_2, cat. Pd/C (10%), DMF, rt *ii)* MeNCO, K_2CO_3, DMF, rt *iii)* 6N HCl, reflux p) *i)* *t*-$BuCH(NMe_2)_2$, DMF, rt *ii))* H_2, cat. Pd/C (10%), DMF, rt q) *i)* NaH, TMSCl, DMF, rt then $Br(CH_2)_4CO_2Et$, rt *ii)* 1N HCl.

Table 2. ***In Vitro*** **and** ***in vivo*** **Activities of NBQX, YM90K and (-)-LY293558 and 8- or/ and 9-Substituted-4-Oxo-Imidazo[1,2-*a*]Indeno [1,2-*e*]Pyrazine Derivatives**



Compd.	R_1	R_2	Receptor affinity[a] IC_{50} (nM)		Anticonvulsant activiy ED_{50} (mg/kg)
			AMPA	NMDA	MES[b] *i.p.*[c] (*i.v.*[d]) DBA/2 *i.p.*[e]
NBQX			140	>10,000	36 (36) 1.3
YM90K			350	10,400	12 (12) 15
(-)-LY293558			600	>10,000	4 (3.4)
1	H	H	760	3000	62
45a	H	8-F	250	7800	-
45b	H	8-Br	2000	>100,000	-
45c	H	8-Cl	16000	>100,000	-
45d	H	8-Me	30.000	>100,000	-
45e	H	8-CO_2H	620	10,000	>80
45f	H	8-CH_2-CO_2H	10.000	10,000	-
45g	H	9-F	900	100,000	-
45i	H	9-NHCONHMe	300	2300	-
45j	H	9-CO_2H	3500	21000	-
45k	H	9-CH_2-CO_2H	89	>10,000	-
46a	H	8-SO_3H	30.000	>100,000	-
46c	H	8-NH_2	5600	22000	-
46d	H	8-$NHCO_2Et$	670	8400	-
46e	H	8-NHCOMe	3250	10000	-
46f	H	8-$NHCONH_2$	1250	>100,000	-
46g	H	8-NHCONHMe	18	100,000	>100
46h	H	8-NHCONHEt	86	10,000	-
46i	H	8-NHCONHPh	620	>100,000	-
46j	H	8-$NHCONHCH_2Ph$	180	100,000	-
47a	-CO_2H	H	150	83	50 20
47b*	-CO_2H	9-CH_2-CO_2H	18	7200	1.2 (0.5) 0.8
47c	-CO_2H	8-CH_2-CO_2H	611	>10,000	16
47d	-CO_2H	9-CHMe-CO_2H	450	4100	19
47e	(*E*)-CH=$CHCO_2H$	9-CH_2-CO_2H	30	>10,000	2.2 (3.8)
47f	-$PO(OH)_2$		13	2360	1 (0.8)
47g**	-$PO(OH)_2$	9-CH_2-CO_2H	150	>10,000	3.5 (2.9)

(Table 2). contd.....

Compd.	R1	R2	Receptor affinity[a] IC_{50} (nM)		Anticonvulsant activiy ED_{50} (mg/kg)
			AMPA	NMDA	MES[b] *i.p.*[c] (*i.v.*[d]) DBA/2 *i.p.*[e]
47h	$-CO_2H$		15	537	2.3 (2)
47i	$-CO_2H$	$9-CH_2-PO(OH)_2$	586	>10,000	-
47j	$-CO_2H$	$9-CH_2-CONHSO_2Ph$	139	2000	-
47k	$-CH_2-CO_2H$	$9-CH_2-CO_2H$	4	2100	2 (0.5)
48	$-CH_2-CH_2-CO_2H$	$9-CH_2-CO_2H$	31	1600	1.9

a: IC_{50} values are mean of at least 3 determinations, each with at least 3 concentrations of tested compound in triplicate. **b**: ED_{50} values (in mg/kg) are defined as the dose which protected 50% of the animals from a tonic convulsion (6 male CD1 mice/dose of compound, with at least 3 doses compared to a group receiving vehicle alone) **c** : in mouse, pre-treatment time: 30 min, vehicle: 1% Tween-80 in water. **d** : in mouse, pre-treatment time: 5 min, vehicle: saline
e : in mouse, pre-treatment time: 30 min, vehicle: saline

*: RPR117824 **: RPR119990

Synthesis of 4-oxo-Imidazo[1,2-*a*]Indeno[1,2-*e*]Pyrazin-8- and 9-Carboxylic (Acetic) Acid Derivatives (45-48)

Encouraged by these results, we attempted to introduce two carboxy functions in positions 2 and 8, 9 or 10 of the imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one ring (Scheme 4). Thus, 9-carboxymethyl-imidazo-indeno-pyrazin-4-one-2-carboxylic acid ((**47b**), **RPR 117824**) displayed nanomolar affinity (IC_{50} = 18 nM, Table 2) 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} = 360 nM). In *in vivo* testing, **RPR 117824** behaves as a powerful blocker of convulsions induced in mice or rats by supramaximal electroshock or chemoconvulsive agents with half maximal effective doses ranging from 1.5 to 10 mg/kg following subcutaneous or intraperitoneal administration (Table 3). In addition, **RPR 117824** exhibited excellent solubility in saline media (≥ 10g/L), enabling intravenous administration [19-20].

Table 3. Duration of Action of Compounds 31, 32b, 39, (+)-35, 47b, 47e-g, 47h, 47k, NBQX, YM90K and (-)-LY293558 in the Mouse, MES Test (*i.v.*)

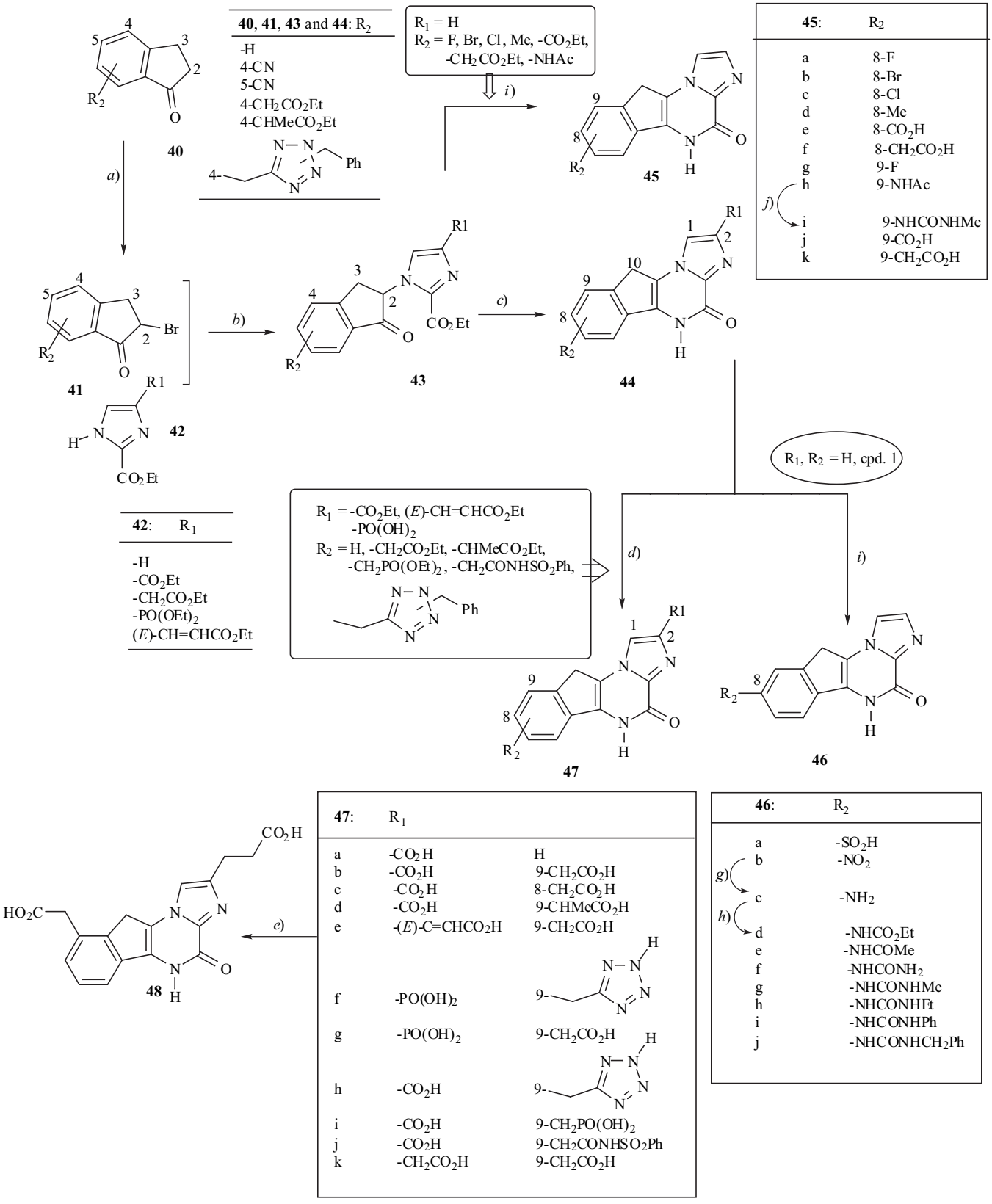
Compd.	Anticonvulsant activiy ED_{50}[a] (mg/kg) (pre-treatment time)			
	5 min	30 min	60 min	180 min
31	1.7	1.7	3	-
32b	10	15	17	-
39	9.5	-	15	-
(+)-35	2.5	5	10	>20
47e	3.8			25.6
47f	0.8			3.5
47h	2			inactive at 40
47k	0.5			28
47b (RPR117824)	0.5	0.95	7.4	25.6
47g (RPR119990)	2.9			3.5
NBQX	36			
YM90K	12	24	40	>40

a: ED_{50} values (mg/kg) are defined as the dose which protected 50% of the animals from a tonic convulsion (6 male CD1 mice/dose of compound, with at least 3 doses plus 1 group receiving vehicle alone).

Then, we decided to replace the carboxylic group of **RPR 117824** in order to investigate further SAR while increasing the chemical diversity (compounds (**45a-f**) [15], [19]; (**46a-j**) [15]; (**47a-k**) [20-21] and (**48**) [20]). Of particular interest was the attempt to introduce a tetrazole ring which is well known to be a bioisostere of a carboxylic acid function and have already led, in several cases, to drastic enhancements of potency, selectivity and/or bioavailability. Thus, we prepared the tetrazole-phosphonic acid derivative (**47f**). A large increase in anticonvulsant potency was obtained by *iv* route compared to **RPR 117824**, indicating that the replacement of the carboxylic function by a tetrazole ring seems to be advantageous from the pharmacodynamic point of view (Table 3).

Synthesis of (45-48)

The 2-bromo indanones (**41**) were obtained from indanones (**40**) using either bromide or pyridinium perbromide monohydrate with a 33-100% yield. Indanones (**41**) reacted with the imidazole-2-carboxylate derivatives (**42**) either under neat phase, in toluene at reflux or using potassium carbonate as a base in acetone at reflux and led to the corresponding 2-substituted indanones (**43**) (33-67% yield). Intramolecular ring closures of (**43**) were carried out either a) using ammonium acetate in glacial acetic acid at reflux or b) according to two-steps synthesis *via* the corresponding 2-carboxamide-imidazole derivatives by an aminolysis reaction followed by intramolecular ring closure reaction using HCl. The 4-oxo-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazines (**44**) and (**45a-h, j,k**) were obtained with moderate to good yields (40-92%). The compound (**45i**) was



Scheme 4. a) Br_2, $CHCl_3$, CH_2Cl_2 or AcOH/HBr (47%), rt or pyridinium perbromide monohydrate, 50°C b) neat phase, 130°C or PhMe, reflux or K_2CO_3, acetone, reflux c) $AcONH_4$, AcOH, reflux d) concd H_2SO_4 or 6-8N HCl or HBr (30-47%), reflux, or 1N NaOH, H_2O-dioxane, rt then 1N HCl, rt e) H_2, 0.3N NaOH, Pd/C (10%), rt then 1N HCl until pH = 7 f) R_2 = -SO_3H: $ClSO_3H$, rt ; R_2 = -NO_2: KNO_3, concd H_2SO_4, rt g) H_2, 0.1N NaOH, cat. Pd/C (10%), rt h) NaH or Et_3N, dioxane or DMF, rt then $ClCO_2Et$ or Ac_2O, rt ; or Et_3N, R-NCO (R = Me_3Si-, Me-, Et-, Ph- or Ph-CH_2-) i) 2.5-N NH_3 or gNH_3, MeOH, rt-reflux then 6-12N HCl, 5°C or NH_4Ac, AcOH, reflux j) 6N HCl reflux then *p*-nitrophenyl-*N*-methylcarbamate followed by 0.5N HCl.

prepared from (**45h**) by the action of HCl and then of *para*-nitrophenyl-*N*-methylcarbamate followed by 0.5N HCl (30% overall yield). Finally, the syntheses of (**47a-j**) were achieved by hydrolysis using either acid (H_2SO_4, HCl or HBr) or basic conditions (NaOH) followed by action of HCl with moderate to good yields (31-86.5%). Hydrogenation of (**47e**) in the presence of a catalytic amount of Pd/C (10%) led to (**48**) with a 50% yield. Compound (**46a**) was obtained from (**1**) by action of chlorosulfonic acid with 86.5% yield, whereas (**46f-j**) were prepared in a 3-steps synthesis by regioselective nitration of (**1**) with potassium nitrate followed by hydrogenation of the nitro group in presence of a catalytic amount of Pd/C (10%), leading to (**46c**) with 42.5% overall yield, and finally condensation of either the corresponding isocyanates with 11-83% yield (**46f-j**), ethyl chloroformate with 26% yield (**46d**) or acetic anhydride with 93% yield (**46e**) in presence of base such as NaH or triethylamine, respectively.

STRUCTURE-ACTIVITY RELATIONSHIPS

The main structure-activity relationships (SAR) of 10*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one series are depicted in the Figure **2**.

In vitro Binding Studies

The affinities for AMPA and NMDA/glycine receptors were evaluated in *in vitro* binding assays on rat cortical membranes preparations using [^{3}H]-AMPA and [^{3}H]-5,7-dichlorokynurenate ([^{3}H]-DCKA) as selective radiolabelled ligands [22]. On the basis of these data, the following structure-activity relationships were highlighted : the position and the nature of the substituents pertaining to the 10*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one cycle (**1**) are crucial (Tables 1 and 2).

Position 8

Introduction of various substituents such as Br or Cl, a methyl group, an electron-withdrawing group such as SO_3H, an electron-donating group such as NH_2 in position 8 decreased the binding at AMPA receptor (2.6-40 fold, (**45b-d**) and (**46a**) *versus* (**1**)) whereas the introduction of a strong electron-negativity source such as a fluorine atom afforded the compound (**45a**) which is up to 3-fold more potent than (**1**). The introduction of a carboxy group (compound (**45e**)) but not a carboxymethyl group (compound (**45f**)) retained the AMPA affinity ((**45e**) *versus* (**1**)), whereas the introduction of an acetylamino (compound (**46e**)) or an ureido group (compound (**46f**)) reduced the binding (~4- and ~2-fold) *versus* (**1**). These compounds exhibited the greatest AMPA affinities but had lower potency for the glycine site (8- to >80-fold). The most significant improvement on the AMPA potency involved the introduction of *N*-alkylated ureido groups such as methyl, ethyl or benzyl which markedly increased the AMPA binding 7-40 fold (compounds (**46g**), (**46h**) and (**46j**)). Within this last sub-series, the more potent urea derivative was (**46g**) which displayed an IC_{50} of 18 nM while it also retained high selectivity *versus* the glycine-binding site (>5000). The phenylureido and ethoxycarboxyamino derivatives (**46d**) and (**46i**) also retained the AMPA affinity *versus* (**1**).

Position 9

Moving the *N*-methyl ureido moiety from 8 to 9 ((**46g**) *vs* (**45i**)) resulted in a 17-fold lower affinity at the AMPA receptor. The same applies to the 9-fluoro derivative (**45g**) but not to the 9-carboxy derivative (**45j**) which decreased 6-fold the AMPA potency ((**45j**) *vs* (**45e**)) but retained selectivity *versus* the glycine-binding site (6-fold). A very interesting improvement of the AMPA affinity was obtained by the introduction of a carboxymethyl group in 9-position of (**1**) (8.5-fold *versus* (**1**) and 100-fold *versus* the corresponding 8-analogue (**45f**).

Through the variations of substituents at positions 8 and 9, it was demonstrated that the most favorable affinities for the AMPA receptors could be obtained with either the *N*-methylureido group at position 8 (**46g**), IC_{50} = 18 nM) or a carboxymethyl group in 9-position (**45k**), IC_{50} = 89 nM). These two disubstituted compounds exhibited lower potency than (**1**) for the glycine site (>30-fold).

Position 2

The most significant improvement on the AMPA potency was observed by the introduction in the position 2 of (**1**) of a carboxy group (compound (**47a**) which increased the binding at both receptor subtypes (5-fold for the AMPA receptor, 36-fold for the glycine/NMDA *versus* the parent compound (**1**)

Positions 2 and 8/ 9

Starting from these interesting results, we decided to introduced both a carboxy group in 2-position and an *N*-methylureido group at position 8 or a carboxymethylgroup in 9-position. The combined introduction of a carboxy group in 2-position and an *N*-methylureido group at position 8 (**37**) or a carboxymethylgroup in 9-position (**47b, RPR117824**) considerably increased the AMPA affinity 17-fold for (**37**) and 8-fold for **RPR117824**. The selectivity observed against the glycine site of the NMDA receptors was > 400-fold.

Further explorations of SAR's in position 2 and 9 were performed by lengthening and/or constraining the carboxyalkyl-like chain. The replacement of the carboxy group in position 2 of **RPR117824** by a carboxymethyl group increased the AMPA affinity (4-fold) with an IC_{50} of 4 nM and retained the selectivity (>500) (**47k**) *versus* **RPR117824**) [23]. The introduction of a carboxymethyl group in position 8 of (47a) decreased by 4-fold the binding affinity at the AMPA receptor ((**47c**) *vs* (**47a**)) as did the introduction of a 1-carboxyethyl group in position 9 of (47a) (3-fold, (**47d**) *vs* **RPR117824**). Pursuing the lengthening of the carboxyalkyl chain by the introduction of either a carboxyethyl group or the unsaturated (*E*)-carboxylidene group at position 2 of **RPR117824** decreased lightly the potency for the AMPA receptor by about 2-fold (**47e**) and (**48**) *versus* **RPR117824**. Note that the same improvement on the AMPA potency was observed in both imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine and 2-carboxy- imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine series (~8-fold, (**45k**) vs (**1**) and **RPR117824** vs (**47a**)) by the introduction in position 9 a carboxymethyl group.

Then, we turned our attention to explore the influence of bioisosteric replacement of carboxylic acids in positions 2

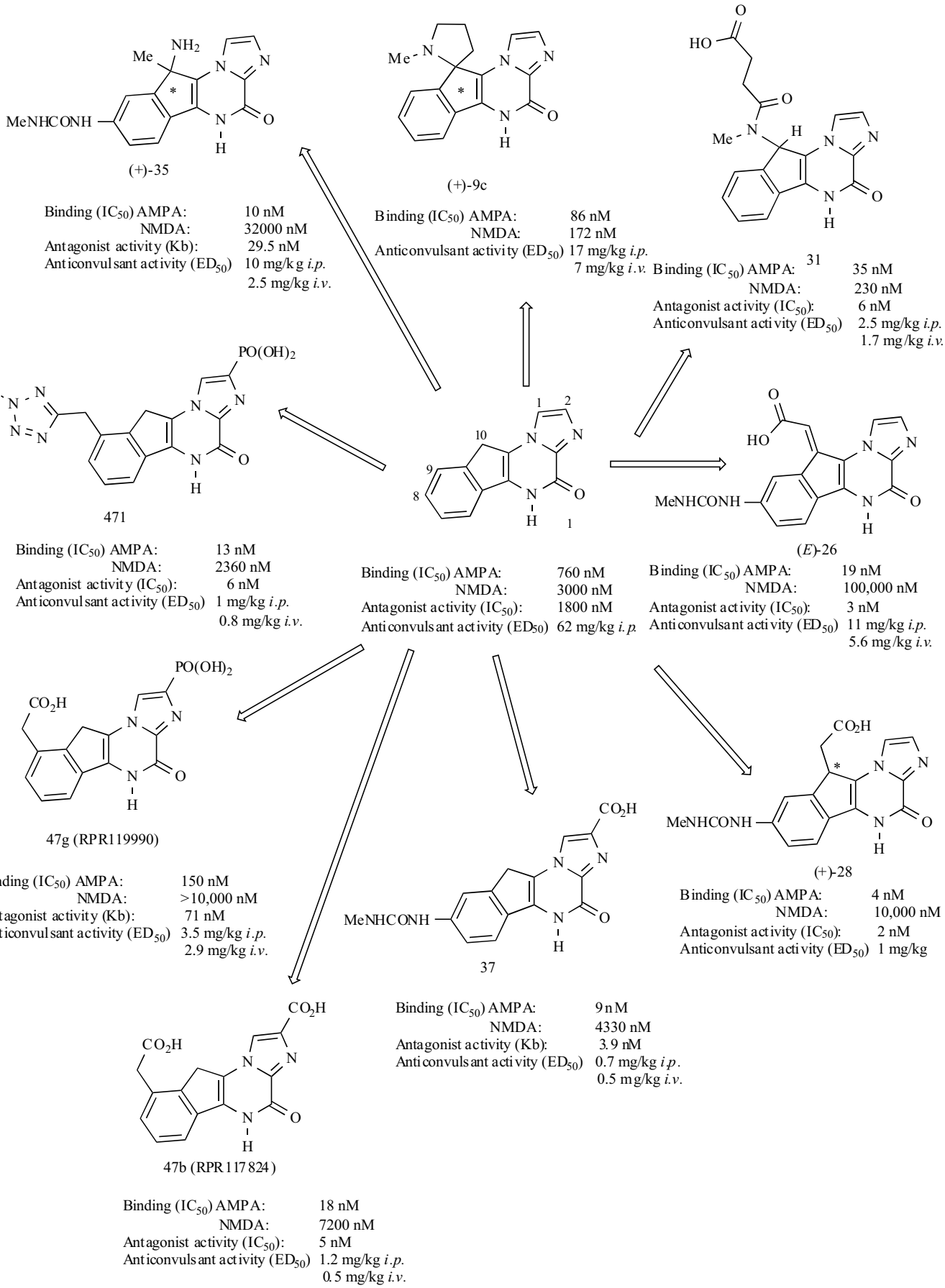


Fig. (2). Main SAR of 10*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one derivatives.

and 9 of parent compound **RPR117824**. Replacement of the carboxylic acid of the chain in 9-position of **RPR117824** by several bioisosteres, that is tetrazole (**47h**), phosphonic acid (**47i**) or *N*-phenylsulfonylcarboxamide (**47j**), was only successful for compound (**47h**) by the introduction of the tetrazolyl moiety which retained the AMPA binding affinity (IC_{50} = 15 nM) but about a 36-fold decrease of selectivity *versus* the glycine/NMDA binding site was observed. The introduction of a phosphonic acid group in position 2 as a substitute for the carboxylic acid of **RPR117824** and (**47h**) induced either a moderate AMPA affinity (IC_{50} = 150 nM, (**47g, RPR119990** [24]) *versus* **RPR117824**) or retained the AMPA affinity (IC_{50} = 13 nM, (**47f**) *versus* (**47h**). The compound (**47f**) still showed reasonable selectivities *versus* the glycine site of the NMDA receptor (>180-fold).

Positions 8 and 10

Starting from the most potent derivative *in vitro* bearing a *N*-methylureido group at position 8 ((**46g**), IC_{50} = 18 nM) a corresponding series of 8, 10-disubstituted acid derivatives has been prepared. Thus, the introduction of a carboxymethyl moiety in position 10 of (**46g**) improved the AMPA affinity by a factor of 20 ((**28**) *versus* (**46g**) and retained the selectivity *versus* NMDA/glycine site (1700-fold), whereas introduction of an *E*-carboxylidene moiety retained the AMPA potency and the selectivity against the NMDA/glycine receptor (**(*E*)-26**) *versus* (**46g**)). The dextrorotatory isomer (**(+)-28**) displayed a ~10-fold greater potency at the AMPA receptors (IC_{50} = 4 nM) than did the levorotatory isomer (**(-)-28**) (IC_{50} = 39 nM), while the selectivity *versus* the NMDA/glycine receptor was more than 250-fold for both isomers. Quite interestingly, the introduction of both an amino group and a methyl group in 10-position of (**46g**) retained the AMPA affinity ((**35**) *vs* (**46g**)). As previously, the dextrorotatory isomer (**(+)-35**) displayed a ~4-fold greater potency at the AMPA receptors (IC_{50} = 10 nM) than did the levorotatory isomer (**(-)-35**) (IC_{50} = 42 nM). Selectivity *versus* the glycine/NMDA receptors was in excess of 3200-fold for the most potent dextrorotatory isomer (**(+)-35**).

Position 10 (di-Substitutions)

Introduction in position 10 of (**1**) of two methyl groups reduced the affinity for the AMPA receptors (6-fold, (**12c**) *versus* (**1**)). Substitution of one of the two methyl groups of (**12c**) by a methylamino group slightly increased the AMPA binding potency (1.3-fold, (**12d**) *versus* (**12c**)).

Position 10 (Spiro-Derivatives)

The most significant improvements on both the AMPA and glycine/NMDA potencies involved the introduction of spiro-derivatives (**9a**) showing similar potency at both receptor subtypes (IC_{50} ~ 250 nM) being 17-fold more potent than (**12c**) for the AMPA receptors. Substitution of the five-membered ring of (**9a**) by a six-membered ring reduced potency (~1.5-4 fold) at both receptor subtypes ((**9b**) *vs* (**9a**)), whereas the displacement of the nitrogen atom in β-position reduced the potency only at the glycine receptor ((**9a**) *vs* (**8**)).

On the basis of these data, we decided to take (**9a**) as the lead compound. Introduction of a methyl group on the nitrogen of (**9a**) retained the potency on both receptor subtypes ((**9c**) *vs* (**9a**)) while introduction of either an ethyl, a *n*-propyl, a benzyl, an amino acetyl, a carboxypropionyl group or insertion of a carboxy group in the α-position of the nitrogen atom of (**9a**) decreased the potency at both receptor subtypes (> 3-fold, (**9d-h**) and (**10**) *vs* (**9a**)). A similar result was obtained by the introduction of various urea groups ((**9i-k**) *vs* (**9a**)), except for the phenyl urea (**9k**) on the glycine site. Replacement of the nitrogen atom of (**9a**) by a carbon atom dramatically decreased the potency on both receptor subtypes ((**12b**) *vs* (**9a**)) as did the introduction of a spiro-cyclopropyl moietie ((**12a**) *vs* (**9a**)).

The good AMPA and glycine-binding site affinities of the racemic (**9c**) prompted us to examine the enantiomers (**(+)-9c**) and (**(-)-9c**). Interestingly, the dextrorotatory isomer (**(+)-9c**) displayed about 50-fold and 7-fold greater potency at the AMPA receptors and glycine site of NMDA receptors respectively than did the levorotatory molecule (**+)-9c**) (IC_{50} [^{3}H]-AMPA = 86 *vs.* 4900 nM, IC_{50} [^{3}H]-DCKA = 172 *vs.* 1160 nM respectively).

Positions 2 and 10

Given the improvement on both receptor subtypes (AMPA and NMDA) by the introduction of a carboxy group in the 2-position of (**1**) (5- and 40-fold, respectively, (**47a**) *vs* (**1**)), (**47a**) has been considered as the lead compound. Introduction of an amino group in position 10 of (**30**) slightly increased the affinity for the AMPA receptors(1.6-fold), but simultaneously decreased that for the glycine-binding site (~2-fold) ((**30**) *vs* (**47a**)). Introduction of an electron-rich heterocycle with electronic effects close to those of amino groups, such as pyrrol-1-yl moiety, increased affinity 1.5-5-fold at both binding sites ((**32b**) vs (**30**)), while a 2-oxo-2,5-dihydropyrrol-1-yl moiety increased only AMPA affinity (4-fold, (**32a**) *vs* (**30**)). Quite interestingly, introduction of a 3-carboxypropionyl chain to the amino function of (**30**) increased the affinity for the AMPA receptors (~3-fold, IC_{50} = 35 nM) while slightly decreasing glycine affinity (~2-fold, (**31**) *vs* (**30**)). The combined introduction of a carboxybutyl chain and a methyl group in position 10 of (**47a**) reduced the affinity at both receptor subtypes (~1.5-fold, (**39**) vs (**47a**)).

In comparison with **NBQX**, **YM90K** and **(-)-LY293558**:

- 10-substituted-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid derivatives (**30**), (**31**) and (**32a,b**) exhibited 1.5- to 26-fold higher affinity at the AMPA receptors. The selectivity observed against the NMDA-glycine site receptors ranged between 1.7 and 32 *versus* at least >30 for **NBQX**, **YM90K** and **(-)-LY293558**.
- **RPR11824** displayed a 7- to 33-fold greater affinity, whereas its bioisosteres (**47f**) and (**47h**) exhibited a 13- to 40-fold higher potency at the AMPA receptors while they retained a good selectivity *versus* the glycine site of the NMDA receptor.
- within the 8-methylureido-imidazo[1,2-a]indeno[1,2-e]pyrazine series, the 2-carboxylic derivative (**37**) exhibited at least a 16-fold higher potency at the AMPA receptors and also maintained the selectivity against the NMDA-glycine site receptors (~480-fold), as did the 10-carboxylidene and the 10-yl-acetic acid

derivatives (**(*E*)-26**) and (**(+)-28**) showing a 7- and 150-fold higher potency with good selectivity.

- the 10,10-amino-methyl derivative (**(+)-35**) exhibited a higher potency (between 14- and 60-fold) at the AMPA receptors and retained selectivity (> 300-fold) against the NMDA-glycine site receptors.
- the spiro derivative (**(+)-9c**) showed a 2-fold, 4-fold and a 7-fold AMPA binding potency to **NBQX**, **YM90K** and **(-)-LY293558**, respectively, but with low selectivity *versus* the NMDA-glycine site receptors (2-fold).

Functional Studies

The functional activity at AMPA receptors was determined using kainate-evoked currents in Xenopus oocytes injected with rat brain mRNAs [25]. The potency of these ligands at AMPA receptors was examined using current response analysis. All compounds exhibited antagonist intrinsic activity against responses elicited by the non-desensitizing AMPA agonist kainate. There was an overall *good correlation* between the IC_{50} in this functional model and the binding affinities. The mechanism of the antagonist activity was studied in more details for the most interesting compounds. Compounds (**37**), **RPR117824**, **RPR119990**, (**(+)-35**) antagonized kainate-induced responses showing Kb's value of 3.9, 5, 29.5, and 71 nM, respectively, while compounds (**(+)-28**), (**(E)-26**), (**31**), (**47f**) and (**39**) exhibited IC_{50}'s values of 2, 3, 6, 6, 22 nM, respectively *versus* **NBQX**, **YM90K** and **(-)-LY293559** having IC_{50}'s values of only 31, 260 and 230 nM, respectively, and (**1**) (Kb = 1800 nM).

***In Vivo* Studies in a Concentration-Dependent Manner**

Anticonvulsant Activity

The anticonvulsant effects of the most potent *in vitro* compounds were evaluated both in normal male CD1 swiss white mice submitted to an electric shock (Maximal Electroshock, MES) following *i.p.* and *i.v.* administrations, 30 and 5 minutes before challenges, respectively [26], and through audiogenic convulsions in DBA/2 mice following *i.p.* administration 30 minutes before challenges [27] (Tables 1 and 2). The ED_{50} value was calculated as the dose of compound which protected 50% of animals [28].

Compound (**1**) penetrates the brain since 30 minutes after *i.p.* administration it protected against supramaximal electroshock (MES) seizures in mice with an ED_{50} of 62 mg/kg and against audiogenic seizures in DBA2 mice with an ED_{50} of 83 mg/kg.

Position 10 (Spiro-Derivatives)

The most active spiro-derivatives *in vitro* (**9a-d**) showed moderate *in vivo* activities (ED_{50} 30-80 mg/kg *i.p.*). Furthermore, the dextrorotatory isomer (**(+)-9c**) was found to be a good anticonvulsant (ED_{50} = 17 mg/kg *i.p*), unlike the levorotatory isomer (**(-)-9c**) which was 5-fold less potent (ED_{50} = 80 mg/kg *i.p*). The isomer (**(+)-9c**) was 4-fold more potent than (**1**) (ED_{50} = 62 mg/kg *i.p*), displayed the same level of potency as **YM90K** (ED_{50} = 12 mg/kg *i.p*), was 4-fold less active than **(-)-LY293558** (ED_{50} = 4 mg/kg *i.p*) and was 2-fold more active than **NBQX** (ED_{50} = 36 mg/kg *i.p*). Compound (**9d**) (ED_{50} = 54 mg/kg *i.p*), showing similar potency then (**9g**) (ED_{50} = 70 mg/kg *i.p*), was about 2-fold less active than (**9c**) thus correlating with the decrease of *in vitro* activity observed for the AMPA binding.

A very interesting result was also obtained with the acidic spiro-derivative (**9h**) which displayed an ED_{50} of 10 mg/kg *i.p*.

In addition, compounds (**9c**) and (**(+)-9c**) showed good anticonvulsant effects in MES tests by *i.v.* route with ED_{50}'s of 10 and 7 mg/kg respectively.

Positions 8 and 10

Compounds (**28**), (**(+)-28**), (**(-)-28**) and (**(*E*)-26**) demonstrated potent *in vivo* activities at doses ≤ 11 mg/kg against both MES-induced convulsions - following *i.p.* and *i.v.* administrations - and audiogenic convulsions in DBA/2 mice (following *i.p.* administration), 5 or 30 minutes before challenges. Compound (**46g**) exhibited low *in vivo* potency in both models (ED_{50} > 100 mg/kg *i.p.*) showing the crucial role of the acid group in position 10 of the 10*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one cycle.

Thus, (**(+)-28**) was found to be a highly potent anticonvulsant (ED_{50} ≤ 1 mg/kg *i.p.*) in both *in vivo* models, unlike the levorotatory isomer (**(-)-28**) which was between 5- and 7-fold less potent than (**(+)-28**) (*ip* route). Compound (**(*E*)-26**) was respectively 10- and 1.7-fold less potent than **(+)-28** by *i.p.* administration in MES and DBA/2 tests respectively.

Compound (**(+)-28**) displayed a higher level of potency than **YM90K** and **(-)-LY293558** (4-12-fold in MES test, 17-fold in DBA/2 test) than the unsubstituted parent compound (**1**) (60-fold in MES test). In addition, (**(+)-28**) and (**(*E*)-26**) demonstrated high anticonvulsant activities by *i.v.* route in the MES test with $ED_{50's}$ of ~ 1-6 mg/kg and this route of administration was facilited by their high solubility in saline solution (7-10 g/L).

Compounds (**35**), (**(+)-35**), (**(-)-35**) demonstrated moderate to potent *in vivo* activity against both type of convulsion. Compounds (**35**) and (**(+)-35**) were found to have greater activity in *in vivo* models (ED_{50} = 4.7-10 mg/kg *i.p.*), unlike the levorotatory isomer (**(-)-35**) which was between 8- and 14-fold less potent in the MES test. Due to a good solubility in physiological saline solution (~2 g/L), (**(+)-35**) exhibited markedly potent activity by *i.v.* route (ED_{50} = 2.5 mg/kg). In addition, (**(+)-35**) exhibited potent anticonvulsant effect in DBA/2 mouse model with an ED_{50} of 1.8 mg/kg *i.p.*

Positions 2 and 10

Compounds (**30**), (**31**), (**32a**), (**32b**), (**39**) and (**47a**) demonstrated moderate to good protective activity *in vivo* against MES-induced convulsions following *i.p*. and *i.v.* administration. (**31**) and (**39**) exhibited the most potent anticonvulsant activity with ED_{50}'s of 2.5 and 10 mg/kg (*i.p*.) and with ED_{50}'s of 1.7 and 9.5 mg/kg by *i.v*. route, respectively. This suggests adequate pharmacokinetics and brain penetration by these routes of administration.

Compound (**31**) was the most potent derivatives in the present series. It displays a ~20-fold higher potency than the

unsubstituted parent compound (**1**) and the 2-carboxylic analogue (**47a**), and a 1.5- to 14-fold greater potency than **NBQX**, **YM90K** and **(-)-LY293558** in the mice MES test by *i.p*. route. More specifically, by *i.v.* route, (**31**), shows a 7- and 13-fold higher potency at 5 min and 1h after dosing, respectively, when compared to **YM90K**, suggesting improvement both in pharmacodynamic and pharmacokinetic properties over this compound. Importantly, compound (**31**), is highly soluble (~ 10g/L) in 0.9% saline solution.

Positions 2 and 8

Compound (**37**) displayed strong anticonvulsant activity in both tests with ED_{50} of 0.7 and 0.5 mg/kg in the MES- and DBA/2-induced convulsions, respectively. This compound showed ~120-fold higher potency than (**1**), and 8- to 70-fold higher potency than **NBQX**, **YM90K** and **(-)-LY293558**. Similary to (**(+)-28**), bearing a carboxymethyl group in its position 10, the introduction of a carboxylic acid moiety in 2 therefore retained both the *in vitro* activity and introduced *in vivo* activities at doses below 1 mg/kg.

Positions 2 and 9

Compounds (**47b, e-h**), (**47k**) and (**48**) demonstrated markedly protective activity *in vivo* against MES-induced convulsions following (*i.p*.) and (*i.v*.) administration with ED_{50}'s ranking between 0.5 and 3.8 mg/kg. These compounds showed high solubilities in saline solution (≥10 g/L) faciliting the *i.v*. administration. Among these compounds, (**47b, RPR117824**) and (**47f**) exhibited strong anticonvulsant activities with ED_{50} of 1.2 and 1 mg/kg *i.p*., respectively, and 0.5 and 0.8 mg/kg *i.v*., respectively. Of note, the two compounds displayed a ~50-fold higher potency than the unsubstituted parent compound (**1**) and the 2-carboxylic analogue (**47a**), and 4- to 35-fold greater *in vivo* activities than **NBQX**, **YM90K** and **(-)-LY293558**.

Duration of Action

The duration of action of several of the most potent compounds in these series was also examined in the mouse MES test (following *i.v.* administration) by increasing the time interval between treatment and electroshock application from 5 min and 180 min (Table 3). Compounds (**31**), (**39**) and **RPR117824** demonstrated the longest durations of action with ED_{50}'s as low as 3, 15 and 7.4 mg/kg, respectively, 1h after administration, and markedly as low as 3.5 mg/kg for **RPR119990** and (**47f**), 3h after administration.

Compared to **RPR117824**, replacement of the carboxy group by a phosphonic acid *in position 2* (**RPR119990**) led to a dramatic increase of potency after a 3-hour pre-treatment (ED_{50} = 3.5 *vs* 25.6 mg/kg). This combines with about a ten fold decrease of affinity for the AMPA receptor, thus the improvement of *in vivo* potency is impressive; it suggests the superiority of the phosphonic over the carboxylic moiety for *in vivo* activity and a a long duration of action, in this series. *In position 9*, replacement of the carboxymethyl group by a 1*H*-tetrazol-5-yl moiety while it did not induce any significant change in (**47h**) compared to to **RPR117824**, but when combined with the introduction of a phosphonic acid in position 2 as in (**47f**) *versus* **RPR119990**, both *in vitro* and *in vivo* potency enhancements were observed giving the very potent and selective AMPA antagonist (**47f**) with a duration of action lasting at least 3 hours. The 8-methyl-ureido-10-amino-10,methyl-imidazo-indeno derivative (**(+)-35**) also showed a long duration of action (ED_{50} < 10 mg/kg when administered 60 min before challenge). This contrasts with **YM90K** where, 3 hours post-administration, the efficacious dose exceeded 40 mg/kg, and **NBQX** which is too rapidly eliminated to estimate an efficious dose in this test 1 h post-administration.

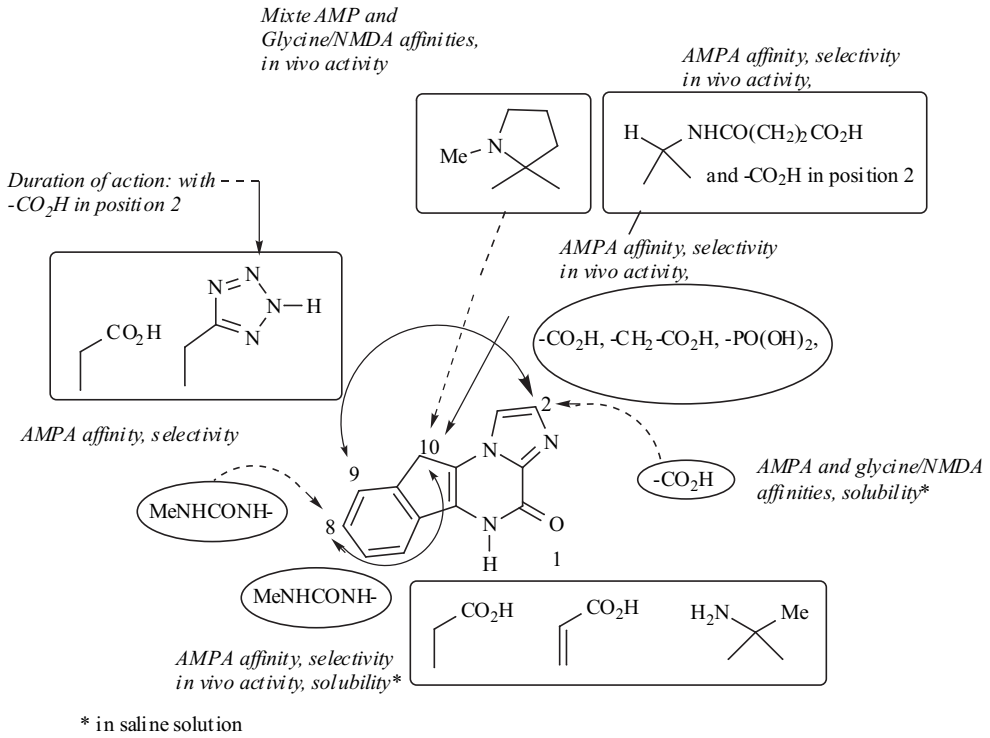


Fig. (3). Main *in vitro* and *in vivo* improvements of 10*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one cycle (**1**).

The Figure **3** summarize the main *in vitro* and *in vivo* improvements of the 10*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one cycle (**1**). Starting from the unsubstituted derivative (**1**), the introduction in the position 2 of a carboxylic acid group increased the binding at both receptors subtypes (AMPA and glycine/NMDA receptors) and the solubility in saline solution whereas the introduction of a simple methylureido moiety in the position 8 strongly increased the AMPA binding and the selectivity *versus* the glycine/NMDA receptors. The introduction of both either a carboxylic acid group (or a carboxymethy or a phosphonic acid group) in the position 2 and a carboxymethy group a tetrazol-5-yl-methyl group in the position 9 increased markedly highly the *in vitro* and the *in vivo* activities as well the selectivity and the solubility in saline solution. The same effects were observed by the introduction of a carboxylic acid group in the position 2 and either a methylureido moiety in the position 8 or a carboxyethyloxoamino group in position 10. The duration of action (MES test) was significantly improved by the introduction of both a phosphonic acid group in position 2 and a carboxymethyl group or a tetrazol-5-yl-methyl group in the position 9. An other very *in vitro* and *in vivo* potent and selective sub-series of compounds was obtained by the introduction of also a methylureido moiety in the position 8 and either a carboxymethyl, a (*E*)-carboxylidene or a both an amino and a methyl group in the position 10. These compounds showed also high solubility in saline solution. (+)-1'-Methyl-spiro{5*H*,10*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-10,2'-pyrrolidine}-4-one is representative of both AMPA and NMDA/glycine antagonist with moderate affinities for these two receptors and with good *in vivo* activities.

Ischemia-Induced Brain Damage and Trauma-Induced Alteration of Brain and Spinal Cord Function

In disease models in rats and gerbils, **(1), RPR117824 and RPR119990** possess significant neuroprotective activity in global and focal cerebral ischemia, and brain and spinal cord trauma.

Models of global ischemia, such as transient bilateral carotid occlusion (BCO) in gerbils, attempt to reproduce the cessation of cerebral blood flow caused by cardiac arrest or stroke with reperfusion. (**1**) (4 × 100 mg/kg i.p. at 0.5h, 4.5h, 24.5h and 28.5h post-occlusion) significantly reduced necrosis in the hippocampal CA1 area induced by the transient ischemia.

Focal ischemia in the rat caused by a permanent occlusion of the middle cerebral artery (MCAO) has similarities to clinical thromboembolic stroke, although reperfusion may also be seen after stroke. (**1**), at 100 mg/kg i.p., administered 0.5 hours *after* MCAO reduced the infarct volume of the cortex by 29% ($p<0.01$). Finally, in a model of spinal cord trauma, treatment with RPR 104151 at 100 mg/kg ip 30' and 24h post crush significantly improved performance on the inclined plane at 24, 48 and 72h post lesion and gave a significantly better neuroscore at 24 and 72h post lesion [12].

RPR 117824 also possesses significant neuroprotective activities in models of global (BCO) and focal cerebral ischemia (MCAO) (MED : 4x8 mg/kg sc and 2x16 mg/kg iv + sc respectively) and in brain and spinal cord trauma (MED : 3x4 mg/kg iv + sc and 2x4 mg/kg +3x8 mg/kg iv + sc respectively) [23].

Activity in an Animal Model of Amyotrophic Lateral Sclerosis

RPR119990 is active in a transgenic mouse model of familial amyotrophic lateral sclerosis (SOD1-G93A mice) where it is able to improve grip muscle strength and glutamate uptake from spinal synaptosomal preparations and prolongs survival with a daily dose of 3 mg/kg sc [24].

CONCLUSIONS

We prepared original series of 4-oxo-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazines derivatives using convergent and versatile synthetic pathways as AMPA antagonists. These compounds displayed moderate to good affinities for the AMPA receptor and *in vivo* activities in anticonvulsant and neuroprotective tests. The best biological activities were obtained with **RPR117824** and **RPR119990**.

RPR117824 (9-carboxymethyl-5*H*,10*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one-2-carboxylic acid) displayed 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 MES 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 *s.c.* or *i.p.* 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 ischemia) and MCAO in rats (focal ischemia), and traumatic brain injury and spinal cord injury models in rats by *i.v.*/s.*c.* administration.

RPR119990 (9-carboxymethyl-5*H*,10*H*-imidazo[1,2-a]indeno[1,2-e]pyrazin-4-one-2-phospho nic acid displaced [^{3}H]-AMPA from rat cortex membranes with a IC_{50} of 150 nM. In *oocytes* expressing human recombinant AMPA receptors, **RPR119990** depressed ion flux with a K_B of 71 nM. **RPR 119990** is a potent anticonvulsant in MES test in mice with ED_{50}'s of 3.5 mg/kg (*i.p.*), 2.9 (*i.v.*) and 2.3 mg/kg (*s.c.*) with a long duration of action (ED_{50} = 3.5 mg/kg *i.v.* when administered 180 min before challenge). This compound was found also active in a transgenic mouse model of familial amyotrophic lateral sclerosis (SOD1-G93A).

Importantly, **RPR117824** and **RPR119990** (as the majority of the compounds of this series) are highly soluble (~ 10g/L) in saline solution, a key feature for the preparation of the *i.v.*-formulations that are needed for the treatment patients suffering from acute neurodegenerative conditions such as cerebral ischemia or central nervous system trauma.

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