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SPIRO-IMIDAZO[1,2-*a*]INDENO[1,2-*e*]PYRAZINE-4-ONE DERIVATIVES ARE MIXED AMPA AND NMDA GLYCINE-SITE ANTAGONISTS ACTIVE *IN VIVO*.

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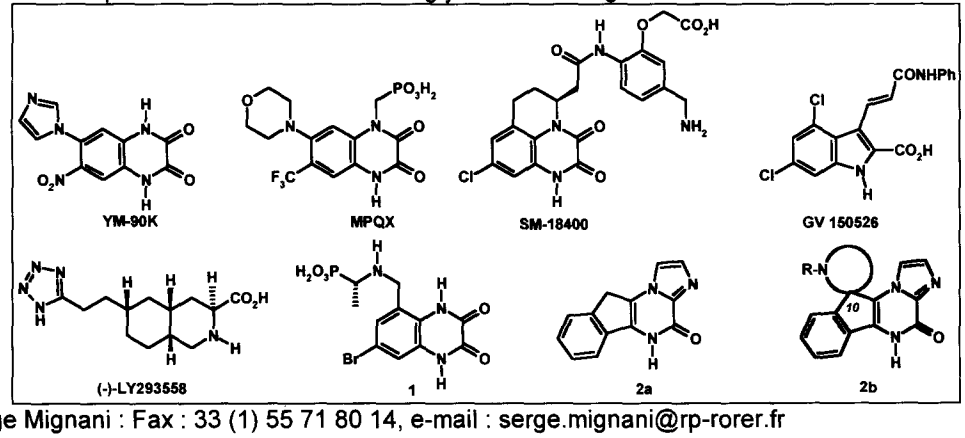
Abstract: *Original spiro-imidazo[1,2-a]indeno[1,2-e]pyrazine-4-one derivatives were synthesised and led to the identification of* **3e** *which showed good affinities for both the AMPA and the NMDA glycine-site receptors, and displayed good anticonvulsant effects after i.p. and i.v. administrations in the electroshock-induced convulsion assay in mice. The corresponding dextrorotatory isomer* **(+)-3e** *was notably more potent than the levorotatory isomer* **(-)-3e** *in in vitro and in vivo assays.*

Excitotoxicity, or the overstimulation of glutamate receptors has been proposed as a pathological phenomenon in a number of neuronal degenerative diseases such as brain ischemia, anoxia and hypoglycemia, traumatic brain and spinal injury, Parkinson's and Huntington's disease.[1] Glutamate excitotoxicity would be mediated by NMDA, AMPA and kainate-preferring glutamate receptor subtypes.[2] Blocking their activation would thus be expected to have a neuroprotective effect. AMPA and NMDA antagonists are of particular interest, and several compounds belonging to various chemical families have been shown to be effective glycine-site NMDA or AMPA antagonists.[3] Representative examples are the AMPA antagonists **YM-90K**[4], **MPQX**[5] and **(-)-LY293558**[6], and the NMDA glycine-site antagonists **SM-18400**[7] and **GV 150526**[8] (Figure 1). To our knowledge, *N*-phosphonoalkyl-5-aminomethylquinoxaline-2,3-dione derivatives such as **1** are the only compounds reported to have high affinities for both the AMPA and the glycine/NMDA receptors.[9] A novel series of competitive AMPA receptor antagonists represented by the 5H, 10H-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one **2a** has been described by us.[10] Compound **2a** displayed significant anti-convulsant properties in

Figure 1: Most prominent AMPA and NMDA glycine-site antagonists.



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mice and rats and activity in models of global cerebral ischemia in the gerbil and focal cerebral ischemia and neurotrauma in the rat.

In this paper, we present original spiro-tricyclic antagonists with the general chemical structure **2b** (Figure 1) such as **3a-p**, **4a-d** and **5a-c** (Scheme 1), their affinities for the AMPA receptor and the glycine-binding site of NMDA receptors, and their anticonvulsant effects in electroshock-induced convulsion assays in mice (MES) following *i.p.* administration as shown in Table 2. SAR of the resulting 10,10-disubstituted imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-ones **2** will be discussed.

Chemistry: The targeted imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one derivatives **3a-p**, **4a-d** and **5a-c**[9] were synthesized from **2a**[10] according to the sequences outlined in Scheme 1.

Synthesis of 3b. Compound **2a** reacted with *tert*-butoxy-bis(dimethylamino)methane followed by hydrolysis using HCl and then reduction by $NaBH_4$ to give **8** with 10% overall yield. Then, this compound was dehydrated using NaOH to give **9** with a 75% yield. As the key step, regioselective [3+2] cycloaddition reaction of **9** with the non-stabilized azomethine ylide **10**[11] obtained *in situ* by action of TFA with *N*-benzyl,*N*-*n*-butoxymethyltrimethylsilylmethylamine, gave **11** with a 41% yield. Finally, *N*-deprotection of **11** was carried out under standard experimental conditions giving **3b** with a 33% yield.

Synthesis of 3a, 3e-h, 3j-l and 3m-o. The *N*-substituted derivatives **3a, 3e-h, 3j-l** and **3m-o** were prepared by treatment of **2a** with *i*$AmNO_2$ in the presence of NaH followed by the action of Zn (powder) in acetic acid giving the key amino intermediate **13** with 6% overall yield. Then, *N*-acetyl deprotection and finally *N*-Boc formation gave **15** with 11% overall yield. Reaction of **15** with 1-chloro-3-bromopropane as electrophile in the presence of NaH afforded **3l** with a 55% yield. Then, **3l** was *N*-deprotected using TFA to give **3a** with a 48% yield. Reductive alkylation of **3a** with formaldehyde and formic acid afforded **3e** with a 54% yield, whereas the *N*-ethylation and *N*-propylation were carried out by action of acetic acid and propionic acid in the presence of $NaBH_4$ giving **3f** and **3g** with 26% and 47% yield respectively. *N*-Benzylation of **3a** was carried out under standard experimental reaction conditions with benzylbromide in the presence of KOH as base giving **3h** with 55% yield. **3j** was synthesized using the condensation of *N*-phthaloylglycine chloride followed by the action of hydrazine with a 10% overall yield. Direct condensation of **3a** with succinic anhydride in acetic acid medium gave **3k** with a 29% yield. The synthesis of the ureas **3m-o** was carried out by the condensation of **3a** with the corresponding isocyanates with good yields (>77%).

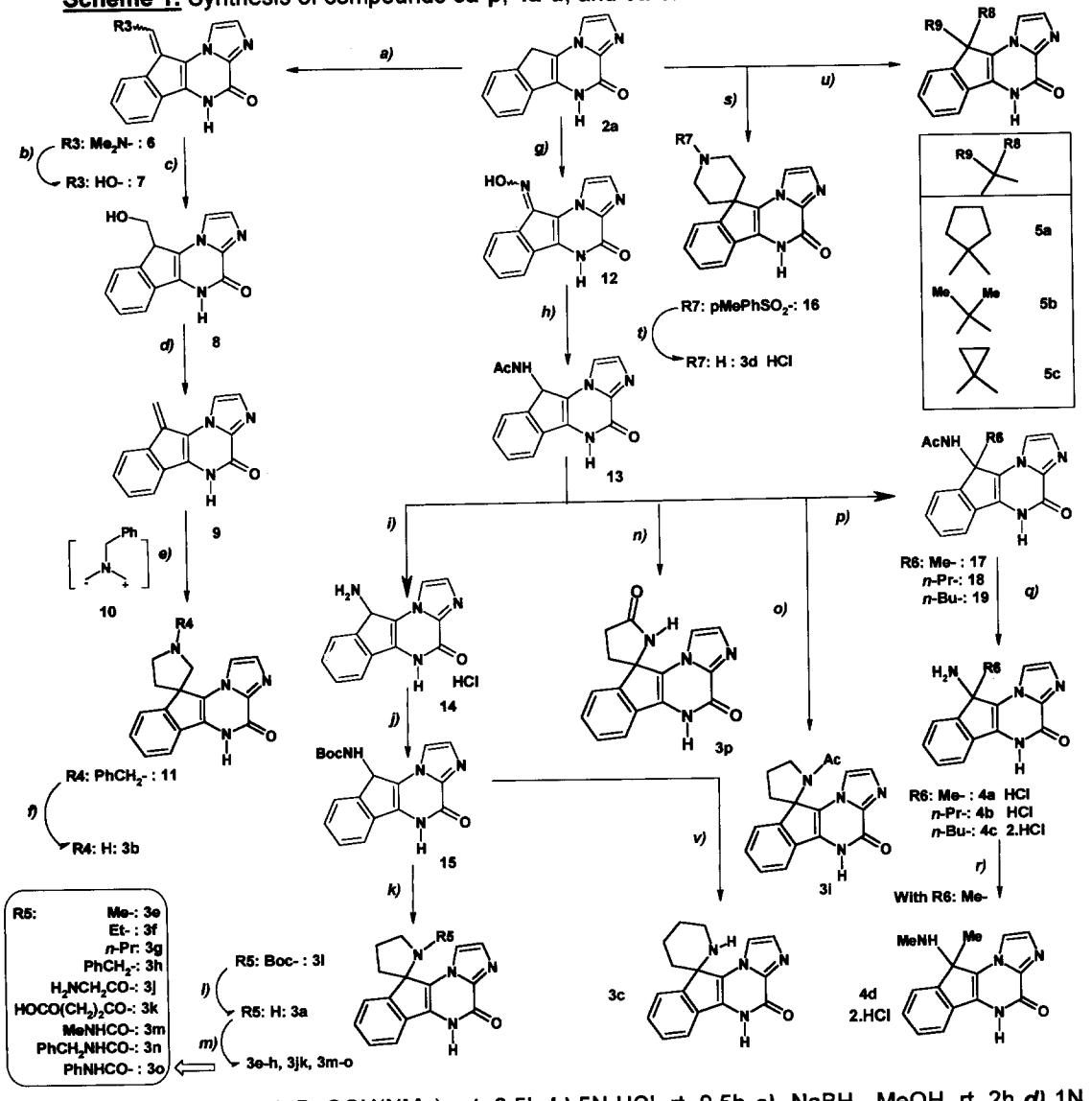
Synthesis of 3c, 3p and 3i : **3c**, **3p** and **3i** were obtained following the same synthetic pathway as the preparation of **3l**. Thus, starting from **15**, condensation of 4-chloro-bromobutane in the presence of NaH followed by *N*-Boc deprotection using TFA gave **3c** with a 60% overall yield, while the spiro-derivatives **3p** and **3i** were obtained with 4% and 11% yield respectively by the condensation of **13** with either methyl 3-bromopropionate or 1-chloro-2-bromoethane, also in the presence of NaH.

Synthesis of 3d: The synthesis of **3d** was carried out by the condensation of **2a** 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 4a-d: **4a-d** was acheived starting from **13.** Alkylation of **13** with methyl iodide, *n*-propyl iodide or *n*-butyl iodide under standard experimental reaction conditions provided the corresponding 10-alkylated derivatives **17**, **18** and **19** with 34.5%, 54% and 45% yield respectively. Then, *N*-deacetylation led to **4a**, **4b** and **4c** with 40%, 59% and 59% yields respectively. The reaction of di-*tert*-butyl dicarbonate with **4a**, followed by *N*-methylation and *N*-deacylation under standard experimental reaction conditions gave **4d** with a 7.5% overall yield.

Synthesis of 5a-c: the spiro-derivatives **5a-c** were prepared from **2a** using catalytic phase transfer conditions in the presence of NaOH and TBAB with 12%, 8% and 31% yield respectively.

Scheme 1: Synthesis of compounds **3a-p**, **4a-d**, and **5a-c**.



Reaction conditions: ***a)*** tBuOCH(NMe)$_2$, rt, 0.5h ***b)*** 5N HCl, rt, 0.5h ***c)*** $NaBH_4$, MeOH, rt, 2h ***d)*** 1N NaOH, MeOH/DMSO, rt, 16h then 1N HCl ***e)*** *n*BuOCH$_2$N(CH$_2$Ph)CH$_2$SiMe$_3$, cat. TFA, DMF, rt, 3h then 60°C, 1h ***f)*** AcOH, H_2 (147 psi), cat. Pd(OH)$_2$/C, 60°C, 3h **g)** NaH, *i*AmNO2, DMSO, rt, 1h ***h)*** Zn, AcOH, 80°C, 2h ***i)*** 2N HCl, reflux, 2h ***j)*** *di-tert*-butyl dicarbonate, Et_3N, DM, 25°C, 20h ***k)*** NaH, Br(CH$_2$)$_3$Cl, rt, 2.5h ***l)*** TFA, 20°C, 2.5h ***m)*** **3e**: HCOH (37%), HCO_2H, 28°C, 1h **3f**: AcOH, $NaBH_4$, 45°C, 16h **3g**: n-$C_3H_7CO_2H$, $NaBH_4$, 45°C, 16h **2h**: $PhCH_2Br$, EtOH, KOH, rt, 12h **2j**: *i) N*-phthaloylglycine chloride, Et_3N, DMF, 25°C, 16h *ii)* $H_2NNH_2.H_2O$, MeOH, reflux, 16h **3k**: succinic anhydride, AcOH, rt, 48h **3m**: MeNCO, DMF, rt, 2h **3n**: $PhCH_2NCO$, DMF, rt, 2h **3o**: PhNCO, DMF, rt, 2h ***n)*** Br(CH$_2$)$_2$CO$_2$Me, NaH, DMSO, rt, 2.5h ***o)*** Cl(CH$_2$)$_2$Br, NaH, DMSO, rt, 2.5h ***p)*** NaH, DMSO, rt, 16h, **17**: MeI, **18**: *n*-PrI, **19**: *n*-BuI, ***q)*** 2N HCl, reflux 1.5-2h ***r)*** *ii) di-tert*-butyl dicarbonate, DMF, 60°C, 20h *ii)* NaH, MeI, DMF, rt, 12h *iii)* TFA, rt, 1.5h then 1.3N HCl, MeOH, rt, 1h ***s)*** *p*-MePhSO$_2$N[(CH$_2$)$_2$Cl]$_2$, NaH, DMSO, rt, 20h ***t)*** HBr (47%), reflux, 5h then 1N HCl ***u)*** TBAB, NaOH, DMSO, rt, 6h, **5a**: Br(CH$_2$)$_4$Br, **5a**: MeI, **5b**: Br(CH$_2$)$_2$Br.

Biological activitiy:

In vitro studies: The affinities for AMPA receptors and the glycine modulatory site on the NMDA receptor were evaluated in an *in vitro* binding assay using [^{3}H]-AMPA[12a] and [^{3}H]-5,7-dichlorokynurenate ([^{3}H]-DCKA) [12b] as selective ^{3}H-ligands on rat cortical membrane preparations. Results for compounds **2a**, **3a-p**, **4a-d**, **5a-c** and **YM-90K** and **(-)-LY-293558** for the AMPA receptors and the glycine-binding site of NMDA receptors are shown in Table 1.

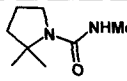
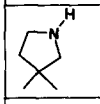
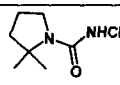
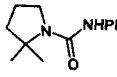
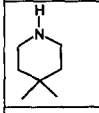
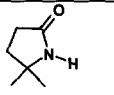
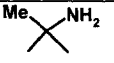
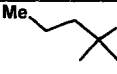
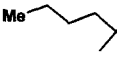
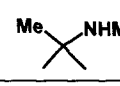
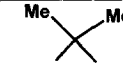
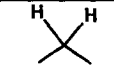
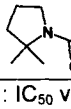
Structure-activity relationships for both receptors (AMPA and NMDA glycine site) were examined: ❶ Introduction in the 10-position of the imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one cycle of **2a** of alkyl groups such as dimethyl, spiro-cyclopentyl or spiro-cyclopropyl moieties reduced the affinity for the AMPA receptors (**5a-c** *vs* **2a**). Substitution of one of two methyl groups of **5b** by an amino group or a methylamino group moderately increased the AMPA binding potency (**4a** and **4d** *vs* **5b**). Substitution of the methyl group of **4a** by a butyl group (**4c** *vs* **4a**) or a propyl group (**4b** *vs* **4a**) increased both AMPA and glycine binding potencies. **4b** was the starting point for the preparation of the corresponding spiro-analogue **3a** which showed similar potency at both receptor subtypes (IC_{50} ~ 250 nM) being at least 3 times more potent than **2a** for the AMPA receptors. ❷ Substitution of the five-membered ring of **3a** by a six-membered ring reduced potency at both receptor subtypes (**3c** *vs* **3a**), whereas the displacement of the nitrogen atom in β-position reduced the potency only at the glycine receptor (**3b** *vs* **3a**). A similar result was obtained in the six-spiro derivative series (**3d** vs **3c**). On the basis of these data, we decided to take **3a** as the lead compound. ❸ Introduction of a methyl group on the nitrogen of **3a** retained the potency on both receptor subtypes (**3e** *vs* **3a**) while introduction of either an ethyl or a *n*-propyl or a benzyl group decreased the potency at both receptor subtypes (**3f**, **3g** and **3h** vs **3a**). ❹ Introduction of either an acetyl (**3i**), an aminoacetyl (**3j**) or a carboxypropionyl (**3k**) chain reduced potency at both receptor subtypes, although this was most dramatic on the glycine-binding site (**3i**, **3j** and **3k** *vs.* **3a**). ❺ Introduction of a Boc function on the nitrogen atom of **3a** reduced potency at both receptor subtypes (**3l** *vs.* **3a**) as did the introduction of various urea groups (**3m-o** *vs.* **3a**) except for the phenyl urea **3o** on the glycine site, and the insertion of a carbonyl group in the α–position of the nitrogen atom of **3a** (**3p** *vs.* **3a**).

The most active compounds **3a-e**, **3k** and **4b** showed similar AMPA binding potency to **YM-90K** and a two fold higher potency than **(-)-LY-293558**. The compounds **3k**, **YM-90K** and **(-)-LY-293558** displayed high discrimination for the AMPA receptors *vs* the glycine-binding site (at least 30 fold) whereas **3a-e** and **4b** showed lower selectivity (1-5 fold).

The good AMPA and glycine-binding site affinities of the racemic **3e** prompted us to examine the enantiomers **(+)-3e** and **(-)-3e** of this compound. The two enantiomers were prepared in optically pure form by HPLC using a column packed with a chiral stationary phase (Chiracel OC phase). Four runs were necessary for the separation, starting from 3.2g of **(+/-)-3e** [mobile phase: ethanol, flow-rate: 30 ml/min, detection: UV (265 nm), column diameter: 60mm]. Enantomeric homogeneity of both enantiomers (>99%) was evaluated by analytical HPLC using the same chiral phase [**(+)-3e :** α_D^{20} = +32.4 (AcOH, c = 0.5) ; **[(-)-3e:** α_D^{20} = -32.0 (AcOH, c = 0.5)]. As shown in Table 1, the dextrorotatory isomer **(+)-3e** 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 **(-)-3e** (IC_{50} [^{3}H]-AMPA = 86 *vs.* 4200 nM, IC_{50} [^{3}H]-DCKA = 172 *vs.* 1160 nM respectively).

In vivo studies: The most active spiro-derivatives *in vitro* [**3abc**, **3ef**, **(+)-3e**, **(-)-3e**] were evaluated for *in vivo* activity in MES[13] assays in mice after *i.p.* administration (1% tween in water) and 30 minutes pretreatment time in comparison with **2a**, **YM-90K** and **(-)-LY29558**. In addition, **3j** and **3k** were selected in order to determine the influence of polar functions such as amino or carboxylic acid groups, whereas **4a** and **4b** were selected in order to determine the influence of a primary amine in position 10 of the imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine-4-one cycle.

Table 1: Binding studies of **2a**, **3a-p**, **4a-d**, **5a-c**, **YM-90K**, and **(-)-LY293558**.

R1, R2 (10)	Cpd	Binding [^{3}H]-AMPA (IC_{50} nM)[a]	Binding [^{3}H]-DCKA (IC_{50} nM)[a]	R1, R2 (10)	Cpd	Binding [^{3}H]-AMPA (IC_{50} nM)[a]	Binding [^{3}H]-DCKA (IC_{50} nM)[a]
	3a	250	280		3m	4000	1700
	3b	200	640		3n	10000	900
	3c	390	1180		3o	7100	130
	3d	380	2000		3p	1500	1700
	3e	200	420		4a	2500	1400
	(+)-3e	86	172		4b	420	520
	(-)-3e	4900	1160		4c	1700	300
	3f	620	530		4d	3400	2740
	3g	4300	470		5a	1600	1400
	3h	10000	1300		5b	4400	1000
	3i	610	560		5c	2300	600
	3j	830	2800		2a	760	3000
	3k	360	10000	YM-90K		350	10400
	3l	>10000	600	(-)-LY293558		600	>10000

a : IC_{50} values (nM) are the mean of at least 3 determinations each with at least 3 concentrations of test compounds in triplicate.

Among these compounds, **3b**, **3a**, **3c** and **3e** showed moderate *in vivo* activities (ED_{50}[14] = >80, 80, 45 and 31 mg/kg respectively). Furthermore, the dextrorotatory isomer **(+)-3e** was found to be a good anticonvulsant (ED_{50} = 17 mg/kg), unlike the levorotatory isomer **(-)-3e** which was 5-fold less potent (ED_{50} = 80 mg/kg). The isomer **(+)-3e** was 4-fold more potent than **2a** (ED_{50} = 62 mg/kg), displayed the same level of potency as **YM-90K** (ED_{50} = 12 mg/kg), and was 4-fold less active than **(-)-LY293558** (ED_{50} = 4 mg/kg). Compound **3f** (ED_{50} = 54 mg/kg), showing similar potency then **3j** (ED_{50} = 70 mg/kg), was about 2-fold less active than **3e** 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 **3k** which displayed an ED_{50} of 10 mg/kg. The lengthening of the alkyl chain of **4a** (ED_{50} = 30 mg/kg) on position 10 decreased 2.5-fold the *in vivo* activity (ED_{50} = 80 mg/kg, **4b** *vs* **4a**). In addition, compounds **3e** and **(+)-3e** showed good anticonvulsant effects in MES tests by *i.v.* route (vehicle: 1 eq. 0.1N HCl, pretreatment time : 5 minutes) with ED_{50}'s of 10 and 7 mg/kg respectively.

In conclusion, **(+)-3e** is representative of an original chemical family of both AMPA and NMDA (glycine) antagonists. It displayed moderate affinities for these two receptors with good *in vivo* activities after *i.p.* and *i.v.* administrations.

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