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Synthesis of Anticonvulsive AMPA Antagonists: 4-Oxo-10-substituted-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic Acid Derivatives

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Abstract—The overstimulation of excitatory amino acid receptors such as the glutamate AMPA receptor has been implicated in the physiopathogenesis of epilepsy as well as in acute and chronic neurodegenerative disorders. An original series of readily water soluble 4-oxo-10-substituted-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid derivatives was synthesized. The most potent derivative **6a** exhibited nanomolar binding affinity ($IC_{50} = 35$ nM) and antagonist activity ($IC_{50} = 6$ nM) at ionotropic AMPA receptor. This compound also demonstrated potent anticonvulsant properties in MES in mice and rats with long durations of action with ED_{50} values in the 1–3 mg/kg dose range following ip and iv administration.

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.[1] Ionotropic glutamate receptors can be divided into NMDA and non-NMDA (AMPA and KA) subtypes on the basis of their preferential affinities for the synthetic excitatory amino-acids *N*-methyl-D-aspartic acid (NMDA) or 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid (AMPA), respectively.[2] 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.[3] As a consequence, the synthesis of specific AMPA antagonists has raised much interest as a source of potential drugs for epilepsy and neurodegenerative diseases.[4]

Various chemical series such as quinoxalines, heterocyclic-fused quinoxalinones, isatinoximes, quinazolines, quinolones, decahydroisoquinoline, and dihydrophthalazine derivatives[5] have been shown to harbor effective AMPA antagonists. Representative examples are **NBQX**,[6] **YM90K**,[7] **YM872**,[8] **NPQX**,[9] (−)-**LY293558**[10] or the non-competitive antagonist **LY300164**[11] (Fig. 1). To date, **YM872** and **LY300164** (talampanel) are evaluated in clinical trials (phase I/II and phase II, respectively) for epilepsy and/or cerebrovascular ischemia.[12]

Based on the anticonvulsant and neuroprotective properties of imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one **1** described previously,[13] we directed our research effort towards the preparation of new 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** which exhibited satisfactory affinities at both the AMPA and the glycine site of the NMDA receptors.[14] By further exploring the structure–activity relationships affording activity at AMPA receptors, we successively discovered 8-methylureido-10-carboxymethyl-(10-carboxylidene)imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one derivatives such as (+)-**3a** and **3b**,[15] 8-methylureido-4-oxo-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid

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derivatives such as **4**,[16] and 9-carboxyalkyl-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic (acetic) acid derivatives such as **5a** and **5b**.[17] All these compounds demonstrated selective affinity and antagonist activity at AMPA receptors and exhibited good anticonvulsant properties in vivo (Fig. 2).

Encouraged by these initial results which demonstrate that the presence of either a methylureido group in position 8 and a carboxylic function in position 10, or a carboxymethyl group in position 9 and a carboxy (or acetic) group in position 2 of the imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one ring **1** provided in vivo activity, we have now attempted to introduce two carboxy functions in positions 2 and 10. The present article summarizes some structure–activity relationships in this new direction.

In this paper, we describe the synthesis, binding and pharmacological properties at AMPA receptors of 4-oxo-10-substituted-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazines **6a**–**c** and **7a**–**f**[18] (Fig. 2, Table 1). We also report their anticonvulsant properties in vivo when administered ip and iv to normal mice submitted to an electric shock (MES).

Chemistry

The targeted 4-oxo-10-substituted-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid derivatives **6a**–**c** and **7a**–**f**[19] were synthesized from 2-bromoindanone **8** according to the sequences outlined in Scheme 1.

Compound **8** reacted with ethyl 4-ethoxycarbonyl-imidazole-2-carboxylate **9**[20] using NaH as a base to afford **10** with a 42% yield. A one-step intramolecular ring closure reaction of **10** was carried out using ammonium acetate in glacial acetic acid at reflux leading directly to **11** with a 38% yield. From **11**, we could access the 4-oxo-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid **12** using standard conditions (6 N HCl) with a 71% yield. Compound **11** also led to the 4,10-dioxo-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid **7a** following a one-pot oxidation-hydrolysis with air-flow under standard reaction conditions (1 N NaOH), although the yield of this reaction was modest (19%).

The 10-hydroxyimino-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid **7b** was prepared by treatment of **11** with *i*AmNO$_2$ in the presence of NaH with a 25% yield. It was then treated with ethyl 5-bromobutyrate, under standard reaction conditions, to give **6b** with a 32% yield, while the treatment of **7b** with Zn powder in ammonium acetate in ethanol and in the presence of ammonia (28%) at reflux followed by the action of 6 N HCl afforded **7c** with a 47% yield. The compound **7c** was the starting material for the preparation of **6a**[21] and **7d**–**g**. Condensation of **7c** with succinic anhydride in the presence of sodium acetate in glacial acetic acid gave **6a** with 66% yield, whereas its condensation with phenylisocyanate using TEA as a base afforded **7d** with a 60% yield.

The synthesis of the 10-pyrrol-1-yl-imidazo[1,2-*a*]indeno-[1,2-*e*]pyrazin-2-carboxylic acid derivatives **7e** and **7f**

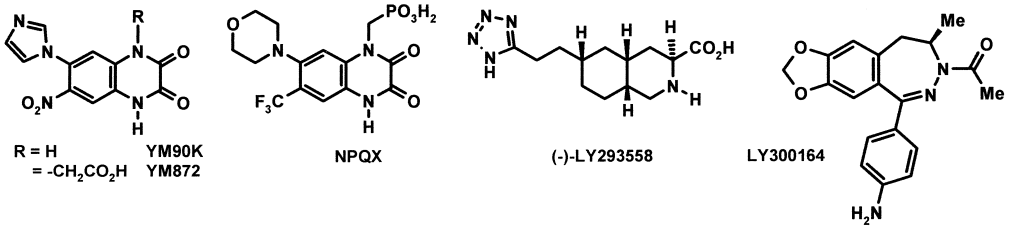


Figure 1.

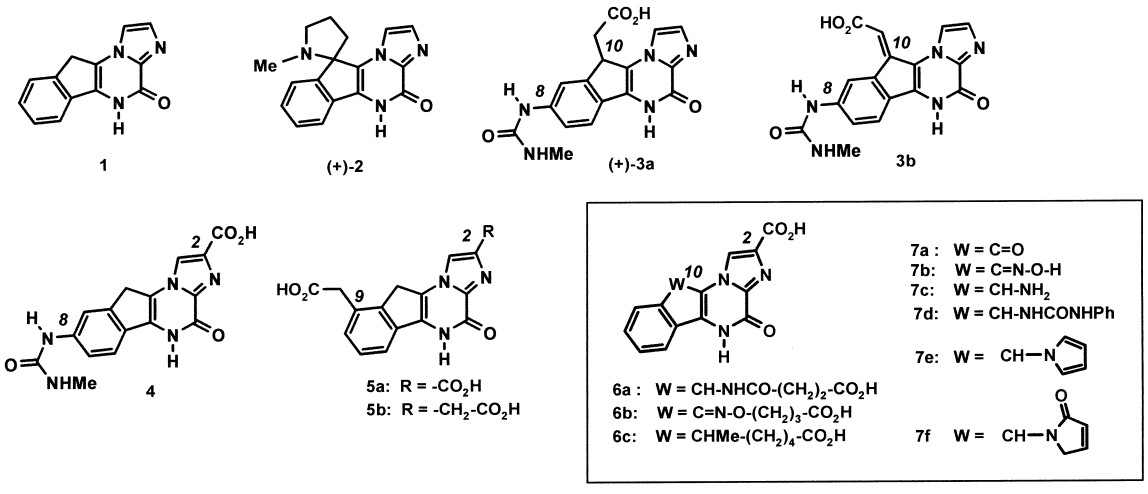
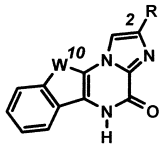
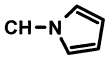
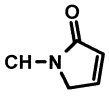


Figure 2.

Table 1. Binding studies and anticonvulsant profile of **1**, **12**, **6a–c**, **7a–f** and **NBQX**, **YM90K** and (−)-**LY293558** following ip and iv administration



R	W	Compound	Receptor affinity[a] IC_{50} (nM)		Anticonvulsant activity MES[b] ED_{50} (mg/kg)		Antagonist activity[e] IC_{50} (nM)
			AMPA	Glycine NMDA	ip[c]	iv[d] (pre-treatment time)	
H	CH–H	**1**	760	3000	62	nd[f]	1800
	CH–H	**12**	150	83	50	nd	29
	$CHNH–CO(CH_2)_2CO_2H$	**6a**	35	230	2.5	1.7 (5 min) 1.7 (30 min) 3 (60 min)	6
	$C=N–O(CH_2)_3CO_2H$	**6b**	44	1400	> 80	nd	5
	$CMe(CH_2)_4CO_2H$	**6c**	242	134	10	9.5 (5 min) 15 (60 min)	22
	C=O	**7a**	580	422	nd	nd	320
	C=N–OH	**7b**	167	593	> 80	nd	130
$–CO_2H$	$CH–NH_2$	**7c**	93	158	19	7 (5 min) 25 (30 min) > 40 (60 min)	130
	CH–NHCONHPh	**7d**	159	18	80		30
		**7e**	55	32	26	10 (5 min) 15 (30 min) 17 (60 min)	60
		**7f**	23	184	20	7.7 (5 min)	—
		NBQX	140	> 10,000	36	36 (5 min)	31
		YM90K	350	10,400	12	6.2 (5 min) 24 (30 min) 40 (60 min)	260
		(−)-**LY293558**	600	> 10,000	4	3.4 (5 min)	233

[a]IC_{50} values are mean of at least three determinations, each with at least three 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 three 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, 30 min and 1h, vehicle: saline.
[e]IC_{50} values (in nM, except for **1**: K_b value in nM from ref. 13) for inhibition of currents generated by 50 μM kainate in *Xenopus* oocytes injected with rat brain mRNA.
[f]nd, not-determined.

were carried out by the condensation of **7c** with 2,5-dimethoxytetrahydrofurane and 2,5-dimethoxy-2,5-dihydrofurane in the presence of sodium acetate and glacial acetic acid leading to **7e** and **7f** with a 41 and 18% yield, respectively.

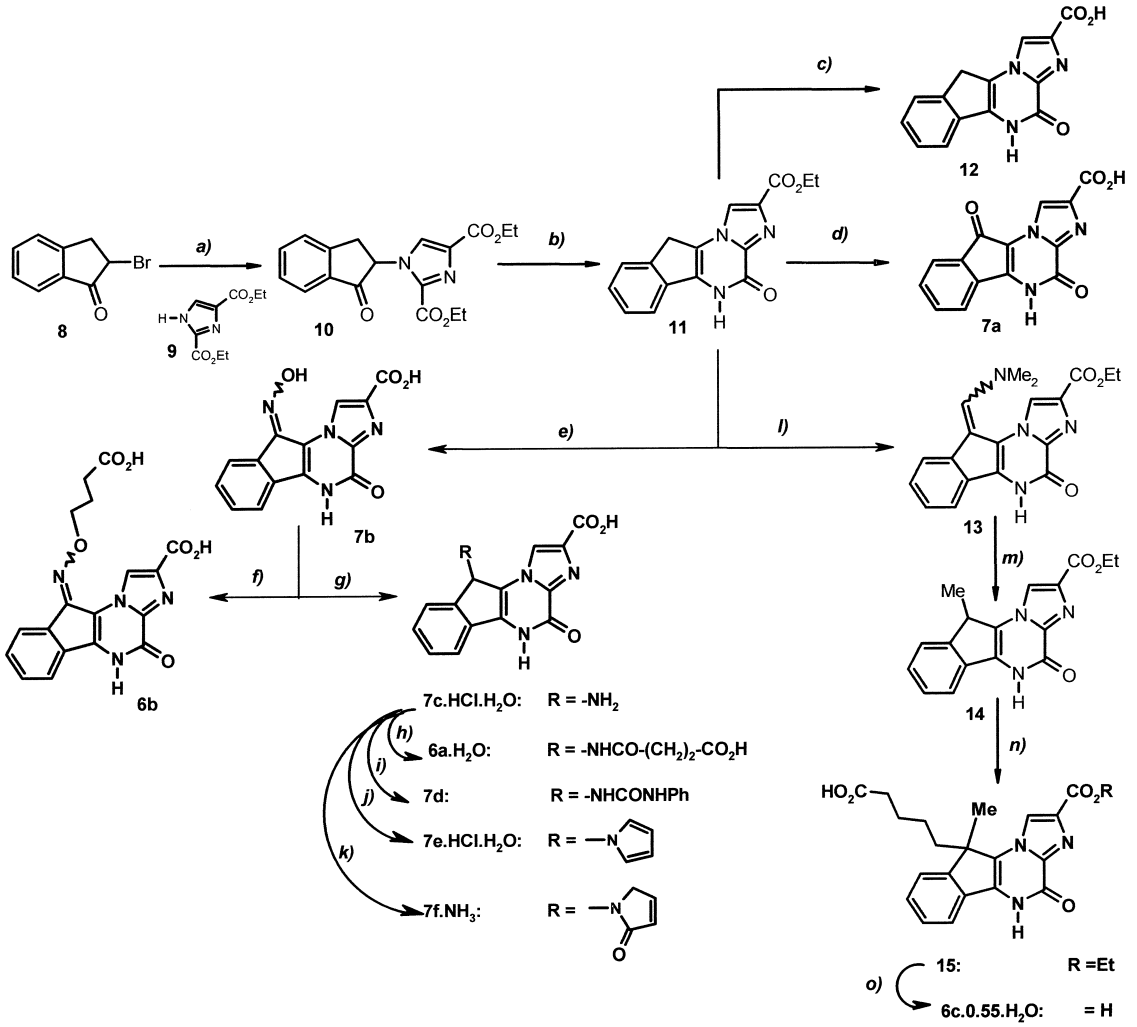
The valeric derivative **6c** was prepared following a four-step synthesis from **11**. The key steps were: (i) action of *tert*-butoxy-bis(dimethylamino)methane leading to the 10-dimethylmethylene moiety (**13**, 53% yield); (ii) formation of the 10-methyl group using hydrogen as the reducing agent in the presence of Pd/C (10%) (**14**, quantitative yield); (iii) alkylation of position 10 of **11** with ethyl 5-bromovalerate in the presence of NaH (**15**, 61% yield); and finally (iv) hydrolysis of the ester moiety of **15** using 1 N NaOH followed by the action of 1 N HCl (**6c**, 19% yield).

Biological Activitiy

In vitro studies

The affinities for AMPA receptor and the glycine site of the NMDA receptor were evaluated on rat cortical membranes using [^{3}H]-AMPA[22] and [^{3}H]-5,7-dichlorokynurenate ([^{3}H]-DCKA)[23] as selective radiolabeled ligands, respectively. The following structure–activity relationship features were identified (Table 1).

Introduction of a carboxy group in the 2-position of **1** increased the AMPA and NMDA binding potency 5- and ~40-fold, respectively (compare **12** vs **1**). Introduction of an amino group in position 10 of **12** slightly increased the affinity for the AMPA receptors 1.5-fold, but simultaneously decreased that for the glycine-binding site ~2-fold (**7c** vs **12**). Introduction in the same



Scheme 1. Synthesis of **6a–c** and **7a–f**: Experimental conditions: (a) NaH, DMF, 15 °C, 45 min then **9**, DMF, rt, 48 h, flash chromatography on silica gel (CH_2Cl_2), 42%; (b) $AcNH_4$, AcOH, reflux, 2 h, 38%; (c) 6 N HCl, AcOH, reflux, 24 h, 71% (d) 1 N NaOH, dioxane, air (flow), rt, 15 h, 19%; (e) NaH, DMSO, *i*$AmNO_2$, rt, 18 h then AcOH, 0 °C, 25%; (f) NaH, DMSO, $Br(CH_2)_3CO_2Et$, rt, 12 h then 6 N HCl, H_2O–MeOH (1:1), 0 °C, 32%; (g) $AcNH_4$, NH_3 (28%), Zn, EtOH, reflux, 5 h then 6 N HCl, 47%; (h) AcNa, AcOH, succinic anhydride, 50 °C, 5 h, 66%; (i) TEA, DMF, PhNCO, rt, 18 h, 60%; (j) AcNa, AcOH, 2,5-dimethoxytetrahydrofurane, reflux, 1.5 h, 41%; (k) AcNa, AcOH, 2,5-dimethoxy-2,5-dihydrofurane, 65 °C, 2 h. Then the mixture was taken to dryness and water was added, flash chromatography on silica gel ($CHCl_3$–MeOH–NH_3 12:6:1), 18%; (l) *t*-$BuCH(NMe_2)_2$, DMF, rt, 5 h, 53%; (m) DMF, Pd/C (10%), H_2 (pressure: 14.7 psi), rt, 15 h, chromatography on Celite (DMF), 100%; (n) NaH, DMF, TMSCl, rt, 0.5 h then $Br(CH_2)_4CO_2Et$, rt, 15 h, then 1 N HCl, 61%; (o) 1 N NaOH, rt, 15 h then 1 N HCl, 19%.

position of an oxo or a hydroxyimino group reduced the affinity for the glycine-binding site preferentially by a factor of 5–7 (**7a** and **7b** vs **12**). Introduction of an electron-rich heterocycle with electronic effects close to those of amino groups, such as a pyrrol-1-yl moiety, increased affinity 1.5–5-fold at both binding sites (**7e** vs **7c**), while a 2-oxo-2,5-dihydropyrrol-1-yl moiety increased only AMPA affinity 4-fold (**7f** vs **7c**). Introduction of a 3-carboxypropionyl chain to the amino function of **7c** increased the affinity for the AMPA receptors ~3-fold while slightly decreasing glycine/NMDA affinity (**6a** vs **7c**). Similarly, introduction of a 3-carboxypropyl group to the hydroxyimino group of **7b** increased the affinity for the AMPA receptors ~4-fold and reduced the affinity for the glycine site 2.5-fold (**6b** vs **7b**). The combined introduction of a carboxybutyl chain and a methyl group in position 10 of **12** reduced the affinity at both receptor subtypes ~1.5-fold (**6c** vs **12**). Finally, the introduction of a phenylurea group on the amino moiety decreased the binding at AMPA receptors ~2-fold while it increased the binding at glycine sites ~9-fold (**7d** vs **7c**).

Compared with **NBQX**, **YM90K**, and (−)**-LY293558**, the 4-oxo-10-substituted-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid derivatives **6a**, **6b**, **7c**, and **7f** exhibited 1.5- to 26-fold higher affinity at the AMPA receptors. The selectivity observed against the glycine site of the NMDA receptors ranged between 1.7 and 32 versus at least >30 for **NBQX**, **YM90K**, and (−)**-LY-293558**. The intrinsic activity of **1**, **12**, **6a–c**, **7a–e**, **NBQX**, **YM90K**, and (−)**-LY-293558** at AMPA receptors was determined using kainate-evoked currents in *Xenopus* oocytes injected with rat brain mRNAs as previously described.[24] All compounds behaved as antagonists in this functional test (Table 1). Their potency correlated significantly with the binding affinities at AMPA receptors (Fig. 3).

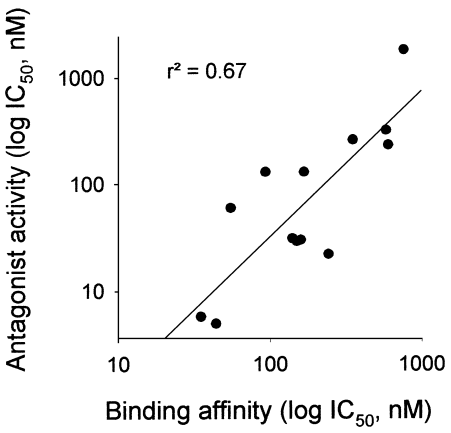


Figure 3. Correlation between antagonist activity against functional responses mediated by rat AMPA receptors expressed in *Xenopus* oocytes. Data are half-maximal inhibitory concentrations determined as in footnote of Table 1 plotted on a log scale.

In vivo studies

As shown in Table 1, compounds **1**, **12**, **6a**, **7c**, **7d**, **7e**, and **7f** demonstrated moderate to good protective activity in vivo against maximal electroshock-induced[25] convulsions in normal male CD1 mice following intraperitoneal (ip) and intravenous (iv) administration. **6a** and **6c** exhibited the most potent anticonvulsant activity with ED_{50}'s of 2.5 and 10 mg/kg by ip route (30 min before challenge) and with ED_{50}'s of 1.7 and 9.5 mg/kg by iv route (5 min before challenge), respectively. This suggests adequate pharmacokinetics and brain penetration by these routes of administration. The duration of action of **6a**, **6c**, **7c**, and **7e** was also examined in the mice MES test by increasing the time interval between treatment and electroshock application from 5 min to 1 h. Compounds **6a** and **6c** demonstrated the longest durations of action with ED_{50} values remaining as low as 3 and 15 mg/kg, respectively, 1 h after iv administration (Table 1).

Compound **6a** was the most potent derivative in the present series. It displays a ~20-fold higher potency than the unsubstituted parent compound **1** and the 2-carboxylic analogue **12**, and a 1.5- to 14-fold greater potency than the literature comparators **NBQX**, **YM90K**, and **(−)-LY293558** in the mice MES test by ip route. More specifically, by iv route, **6a** shows a 3.5- and 13-fold higher potency at 5 min and 1 h after dosing, respectively, when compared to **YM90K**, suggesting improvement both in pharmacodynamic and pharmacokinetic properties over this compound. The testing of **6a** in the MES assay was extended to rats. In this species, the compound displayed ED_{50}'s of 1.8, 1.1, and 1.6 mg/kg for pre-treatment times of 5 min, 30 min, and 1 h, respectively. This contrasts with **NBQX** which is too rapidly eliminated to estimate an efficacious dose in this test 1 h post-administration. Compound **6a** displays 1.7-fold higher potency versus **YM90K** following iv route. Importantly, compound **6a** is highly soluble (~10 g/L) in 0.9% saline solution, a key feature for the preparation of the iv formulations that are needed for the treatment of patients suffering from acute neurodegenerative conditions such as cerebral ischemia or trauma.

In conclusion, this study reports a novel series of readily hydrosoluble heterocyclic-fused 4-oxo-10-substituted-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid derivatives exemplified by compound **6a**. This compound strongly binds the AMPA receptor and antagonizes its function at nanomolar concentrations. Compound **6a** is a potent anticonvulsant in vivo following both ip and iv administration endowed with prolonged duration of action following iv administration with respect to reference comparators. The anticonvulsant profile of this compound confirms the involvement of AMPA receptors in epileptogenic processes and highlights the interest of new AMPA antagonists as potential treatments against epilepsy and acute or chronic neurodegenerative diseases.

Acknowledgements

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