Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1133–1137

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

4,10-Dihydro-4-oxo-4*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic Acid Derivatives: Highly Potent and Selective AMPA Receptors Antagonists with In Vivo Activity

Jean-Marie Stutzmann, Georg Andrees Bohme, Alain Boireau, Dominique Damour, Marc Williams Debono, Arielle Genevois-Borella, Assunta Imperato, Patrick Jimonet, Jeremy Pratt, John C. R. Randle,† Yves Ribeill,‡ Marc Vuilhorgne and Serge Mignani*

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

Received 21 January 2000; accepted 20 March 2000

Abstract—A novel series of 2-substituted-4,5-dihydro-4-oxo-4*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine derivatives was synthesised. One of them, **4e**—a highly water soluble compound—exhibited a nanomolar affinity and demonstrated competitive antagonist properties at the ionotropic AMPA receptors. This compound also displayed potent anticonvulsant properties against electrically or sound-induced convulsions in mice after systemic administration, thus suggesting adequate brain penetration.

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 ionotropic 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 their 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 more recently recognised that AMPA receptors may also be significantly involved in neuronal death.[1] As a consequence, the synthesis of specific AMPA antagonists has raised much interest as source of potential drugs for cerebral ischemia or epilepsy.[2]

AMPA antagonists have been already obtained from various chemical series such as quinoxalines heterocyclic-fused quinoxalinones, isatinoximes, quinazolines, quinolones and decahydroisoquinoline.[3] Representative examples are NBQX, YM90K and (−)-LY293558, as well as the more recently described ZK200775[4] (Fig. 1).

Based on the initial anticonvulsant and neuroprotective properties of imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one **1**,[5] important efforts led to the preparation of two original series of active compounds. Spiro-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one derivatives such as **(+)-2** which exhibited affinities for both the AMPA receptors and the glycine site of the NMDA receptor,[6] while 8-methylureido-10-substituted-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one derivatives such as **(+)-3** only demonstrated a high affinity for the AMPA receptors[7] (Fig. 1, Table 1).

As part of our program aimed at the development of potent excitatory aminoacid antagonists, we now report the synthesis of 2-substituted-4,10-dihydro-4-oxo-4*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine derivatives **4a**–**h**.[8a,b] Also, the affinities for AMPA and glycine/NMDA were evaluated as well as anticonvulsant activity against MES and sound induced seizures (Scheme 1, Table 1). The 8-methylureido-imidazo-indenopyrazine-2-carboxylic acid **4e** displayed a nanomolar affinity and was found to be a competitive antagonist (Fig. 2). This compound is a potent anticonvulsant when administered by intraperitoneal

*Corresponding author. Tel.: +33-1-5571-8305; fax: +33-1-5571-8014; e-mail: serge.mignani@aventis.com

†Present address: Vertex Pharmaceuticals Incorporated, Cambridge, MA 02139-4242, USA.

‡Present address: Aventis Crop Science, Research Triangle Park, NC 27709, USA.

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00181-5

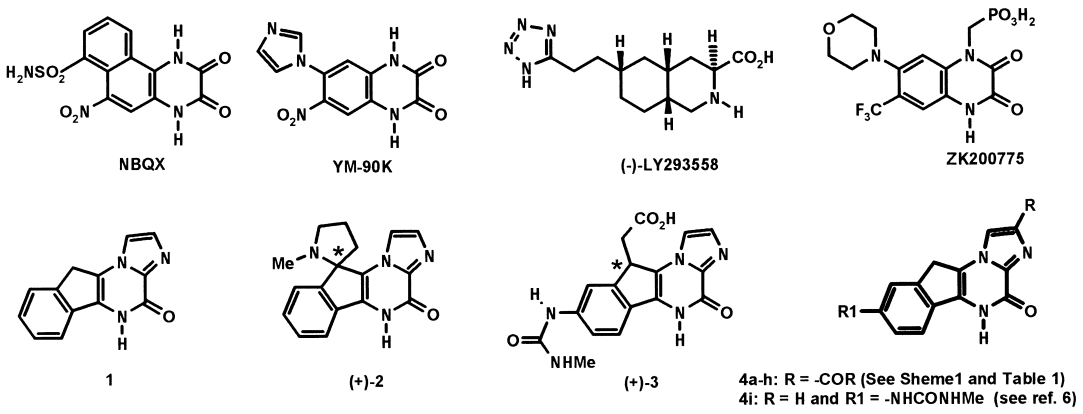


Figure 1.

Table 1. Binding studies, in vitro pharmacology and anticonvulsant profile by intraperitoneal route of **1**, (+)-**2**, (+)-**3**, **4a**–**h**, **NBQX, YM90K** and **(−)-LY293558**

			Receptor affinity[a]		Antagonist activity[b]	Anticonvulsant activity	
R	R1	Compound	AMPA	NMDA		MES[c]	DBA/2[d]
$-CO_2H$	H	**4a**	150	83	30	50	20
$-CO_2H$	-F	**4b**	60	455	25	56	nt
$-CONH_2$	H	**4c**	476	730	1250	>80	nt
$-CO_2H$	$-NH_2$	**4d**	473	6900	236	80	nt
$-CO_2H$	-NHCONHMe	**4e**	9	4330	Kb: see Figure 1	0.7	0.5
$-CO_2Et$	H	**4f**	210	198	350	>80	>80
$-CO_2Et$	-F	**4g**	55	nt	nt	>80	nt
$-CO_2Et$	-NHCONHMe	**4h**	9	10,000	6	>80	>20
H	H	**1**	760	3000	1800	62	nt
		(+)-2	86	172	890	17	nt
		(+)-3	4	10,000	2	1	0.9
		NBQX	140	>10,000	31	36	13
		YM90K	350	10,400	260	12	15
		(−)-LY293559	600	>10,000	230	4	nt

[a]IC_{50} values (in nM) are mean of at least three determinations, each with at least three concentrations of tested compound in triplicate.
[b]IC_{50} values (in nM, except for **1**: Kb value in nM from ref 5) for inhibition of currents generated by 50 μM kainate in *Xenopus* oocytes injected with rat brain mRNA.
[c]ED_{50} values (in mg/kg) are defined as the dose which protected 50% of the animals from a tonic convulsion (six male CD1 mice/dose of compound, with at least three doses plus one group receiving vehicle alone [13] (vehicle: 1% tween in water), pretreatment time: 30 min.
[d]ED_{50} values (mg/kg) are defined as the dose which protected 50% of the animals from tonico-clonic convulsions[14] (vehicle: 1% tween in water), pretreatment time: 30 min.

route to normal mice submitted to an electric shock (maximal electroshock, MES) or genetically seizure prone DBA/2 mice submitted to sound. Compound **4e** was active in both models at doses below 1 mg/kg (Table 1).

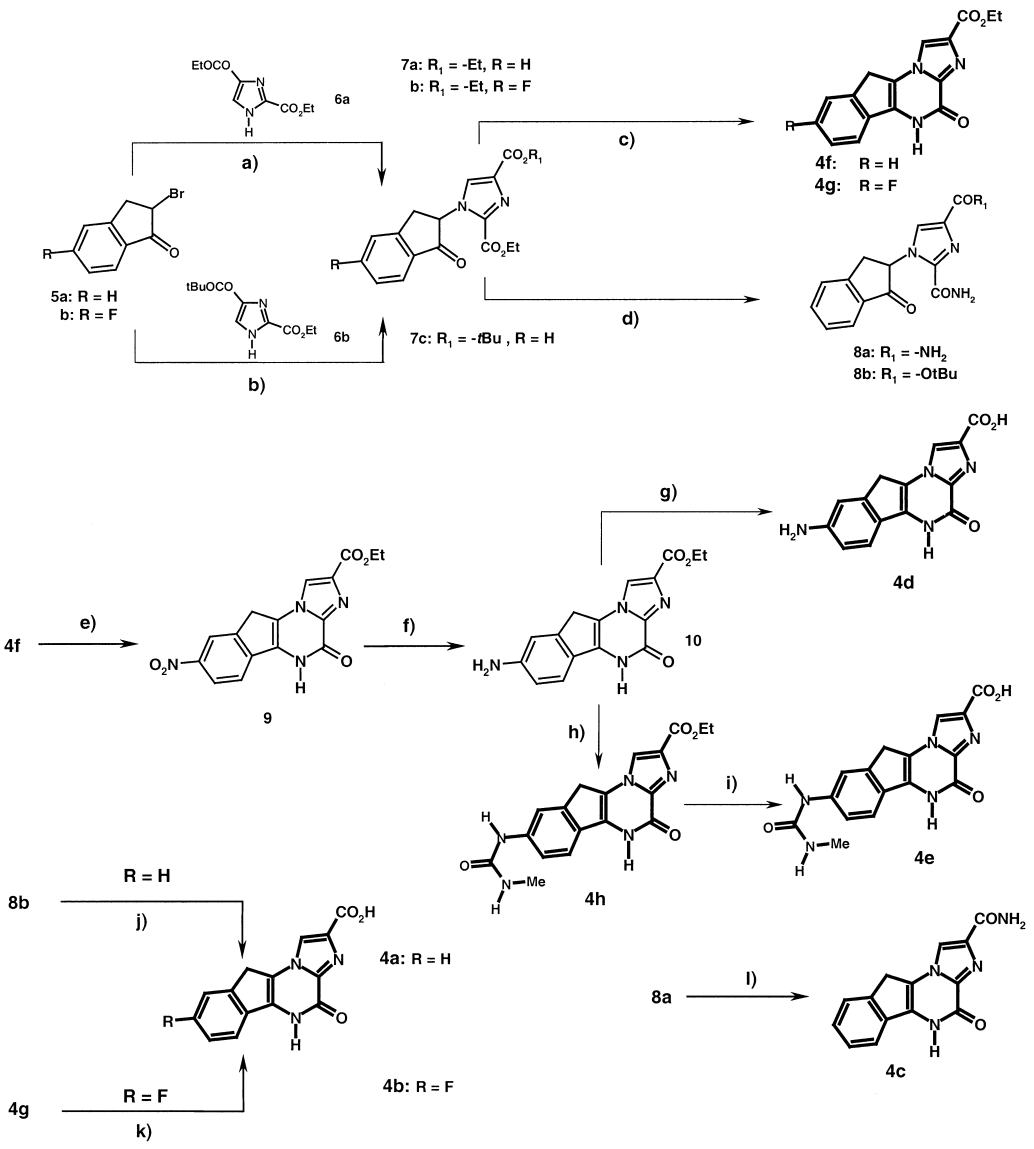
Chemistry

The targeted 2-substituted-4,10-dihydro-4-oxo-4*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazine derivatives **4a**–**h** were prepared from the 2-bromo indanones **5a**,**b** according to the sequences outlined in Scheme 1. Our synthetic approaches to the tetracyclic derivatives **4a**–**h** started with the condensation of either ethyl 4-ethoxycarbonyl-imidazole-2-carboxylate **6a**[9a,b] or ethyl 4-*tert*-butyloxy-carbonyl-imidazole-2-carboxylate **6b**[10] with the 2-bromo indanones **5a**,**b** using NaH or K_2CO_3 as base to afford **7a**, **7b** and **7c** with a 40–50% yield. Then, the carboxamide derivatives **8a**,**b** were easily obtained from the corresponding ester derivatives **7a** and **7c** by ammonolysis reaction with ammonia gas in methanol with a 70 and 80% yield, respectively.

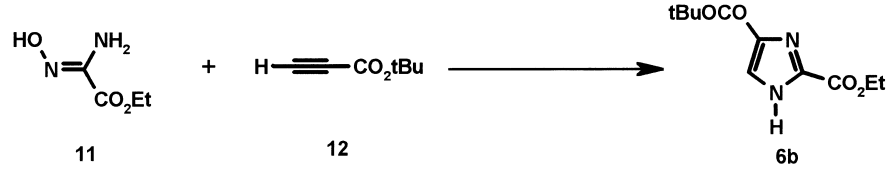
Synthesis of 6b

Compound **6b** was prepared following a procedure similar to that to obtain **6a**[9b] in a one-step synthesis by the condensation-cyclization reaction of ethyl α-amino-oximinoacetate **11** with *tert*-butyl propiolate **12** with a 27.5% yield according to Scheme 2.

Synthesis of 4f–g and 9–10. Intramolecular ring closure reactions of **7a** and **7b** were carried out using ammonium acetate in glacial acetic acid leading directly to **4f** and **4g** with 38 and 16% yield, respectively. With **4f** in hand, we turned our attention to the synthesis of the ethyl 8-amino-4,10-dihydro-4-oxo-4*H*-imidazo[1,2-*a*] indeno[1,2-*e*] pyrazin-2-carboxylate **10** which was obtained by the regioselective nitration of **4f** with potassium nitrate in concentrated sulfuric acid followed hydrogenation of the nitro group in presence of a catalytic amount of Pd/C



Scheme 1. Synthesis of compounds **4a**–**h**. Experimental conditions: (a) **7a**: **6a**, NaH, DMF, 15 °C, 45 min then **5a**, CH_2Cl_2, rt, 48 h, flash chromatography on silica gel (CH_2Cl_2), 42%; **7b**: **6a**, **5b**, K_2CO_3, acetone, reflux, 2 h, flash chromatography on silica gel (cyclohexane:EtOAc 30:70), 47%; (b) **6b**, NaH, DMF, 15 °C, 45 min then **5a**, CH_2Cl_2, rt, 48 h, flash chromatography on silica gel (cyclohexane:EtOAc 50:50), 49%; (c) **4f**: **7a**, AcOH, NH_4Ac, reflux, 2 h, 38%; **4g**: **7b**, AcOH, NH_4Ac, reflux, 2h, 16%; (d) **8a**: **7a**, MeOH, NH_3 (gas), 30 min then rt, 48h, 70%; **8b**: **7c**, MeOH, NH_3 (gas), 30 min then rt, 20 h, 80%; (e) **4f**, concd H_2SO_4, −5 °C to 0 °C, 30 min then potassium nitrate, 0 °C (30 min) to rt (12 h), 96% (f) H_2 (pressure of hydrogen: 28 psi), cat.Pd/C (10%), DMF, rt, 24 h, 58%; (g) 6N HCl, reflux, 24 h, 59% (h) MeNCO, DMF, rt, 12 h, 46%; (i) 1N NaOH, dioxane, rt, 12 h then 1N HCl until pH = 1 and precipitation, 67%; (j) AcOH, reflux, 7 h, 83%; (k) 6N HCl, reflux, 16 h, 39.5% (l) AcOH, reflux, 7h, 54%.



Scheme 2. Synthesis of **6b**. Reaction conditions: TEA, xylene, rt, 20 h, flash chromatography on silica gel (cyclohexane:AcOEt 50:50), 27.5%.

(10%) under standard reaction conditions with a 56% overall yield.

Synthesis of 4b, 4d, 4e and 4h. Hydrolysis of the ester **10** and **4g** using 6 N HCl at reflux gave the corresponding carboxylic acid derivatives **4d** and **4b** with 59 and 39.5% yield, respectively. The urea derivative **4h** was obtained (46% yield) by reacting **10** with methylisocyanate in DMF. Hydrolysis of the ethyl 8-methylureido-2-carboxylate **4h** afforded its corresponding 2-carboxylic acid **4e** (67% yield).

Synthesis of 4a and 4c. The one step intramolecular cyclization-hydrolysis reactions of **8b** and **8a** were carried out using acetic acid at reflux. This led directly to **4a** and **4c** with an 83 and 54% yield respectively.

All new compounds have been characterised by ^{1}H NMR, IR and Mass spectroscopy, and the target derivatives **4a–h** also gave satisfactory elemental analyses (C, H, N).

Biological activitiy

In vitro studies:

The affinities for AMPA and glycine/NMDA receptors were evaluated in in vitro binding assays using [^{3}H]-AMPA[11] and [^{3}H]-5,7-dichlorokynurenate[12] ([^{3}H]-DCKA) as selective ^{3}H-ligands on rat cortical membrane preparations. Results for compounds **1**, **(+)-2**, **(+)-3**, **4a–h**, **NBQX**, **YM90K**, and **(−)-LY293559** for the AMPA and the glycine/NMDA receptors are shown in Table 1.

On the basis of the binding data, the following structure–activity relationships were observed: Introduction in the position 2 of the imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one cycle **1** of either an ethoxycarbonyl or a carboxylic acid groups increased the binding at both receptors subtypes (4-fold for the AMPA receptors, 20-fold for the glycine/NMDA receptors; **4a** and **4f** versus **1**), whereas the introduction of a carbamoyl moiety left the affinity for the AMPA receptors unchanged while it increased (~4-fold) the affinity for the glycine site of the NMDA receptors (**4c** versus **1**).

Introduction in the position 8 of **4a** and **4f** of a fluorine atom afforded the compounds **4b** and **4g** which are up to 2.5- and 4-fold more potent at the AMPA receptors than **4a** and **4b**, respectively, suggesting that position 8 of the imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-4-one cycle to be critical for obtaining a high affinity. Given these results and the highest affinity for the AMPA receptor already obtained by the introduction in position 8 of **1** of a methylurea moiety (compound **4i**, $IC_{50} = 18$ nM[7]), introduction of the same group for **4a** and **4f** increased highly the potency for the AMPA receptors ($IC_{50} = 9$ nM) while retaining the observed selectivity against the glycine/NMDA receptors ($IC_{50} = 4000–10{,}000$ nM, **4e** and **4h** versus **4a** and **4f**).

In comparison with **NBQX**, **YM90K** and **(−)-LY293559**, the fused 8-methylureido-indenopyrazinone derivatives **4e** and **4h** exhibited between a 15- to 70-fold higher potency at the AMPA receptors and retained the selectivity (>400-fold) against the glycine site of the NMDA receptors. In addition, **4e** and **4h** displayed a similar level of potency for the AMPA receptors compared to compound **(+)-3**, and were 10-fold more potent for the AMPA receptor than **(+)-2**.

The pharmacology of these ligands at AMPA receptors was routinely examined using electrophysiological responses. All compounds in the series exhibited antagonist intrinsic activity against responses ellicited 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 (Table 1). The mechanism of the antagonist activity was studied in some details for the most promising compound **4e** (Fig. 2).

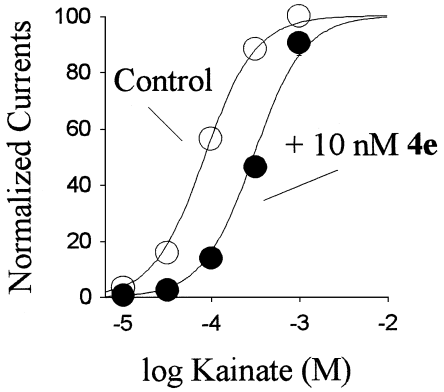


Figure 2. Antagonist activity of compound **4e** against functional responses mediated by AMPA receptors in *Xenopus* oocytes. Inward-currents were recorded in voltage-clamped oocytes injected with rat brain mRNA as per previously described methods.[15] In the presence of 10 nM of compound **4e**, a parallel rightward shift of the kainate concentration-response curve was observed, indicating competitive antagonism at AMPA receptors. The equilibrium constant Kb calculated from the concentration-ratio was 3.9 nM.

In vivo studies. Compounds **4a**,**b**,**d**,**e** demonstrated in vivo activities against both MES-induced convulsions in normal mice and sound induced convulsions in DBA/2 (compounds **4a** and **4e**) mice following intraperitoneal (ip) administration 30 min before challenge. Among these compounds, **4a**,**b**,**d** exhibited moderate anticonvulsant potency ($ED_{50} = 50–80$ mg/kg), whereas **4e** displayed strong anticonvulsant activity in both tests with ED_{50} of 0.7 and 0.5 mg/kg in the MES and DBA/2 assays, respectively. This compound showed a ~120-fold higher potency than its unsubstituted parent compound **1**, and a 8- to 70-fold higher potency than **NBQX**, **YM90K** and **(−)-LY293559**. Similary to **(+)-3**, bearing a carboxymethyl group in its 10-position, the introduction of a carboxylic acid moiety in 2 therefore retained both the in vitro activity at AMPA receptors and introduced in vivo activities at doses below 1 mg/kg. Compound **4e** is 34-fold more potent than the spiro-derivative **(+)-2**. On the other hand, replacement of the carboxylic acid group of **4a** with the carboxamide one or esterification of **4a**, **4b** and **4e** conferred no in vivo activity to the corresponding compounds at doses as high as 80 mg/kg.

In conclusion, the 8-methylureido-4,10-dihydro-4-oxo-4*H*-imidazo[1,2-*a*]indeno[1,2-*e*]pyrazin-2-carboxylic acid **4e** possesses one of the highest affinities for the AMPA subtype of glutamate receptors with a IC_{50} of 9 nM and exhibits potent anticonvulsant effects following systemic administration ($EC_{50} < 1$ mg/kg), suggesting adequate passage of the blood-brain barrier. The sodium salt is highly water-soluble (1 mmol/L, pH = 7–8). The log *P* value of compound **4e** being lower than usually expected to allow brain penetration (*C* log $P = 0.92$), it suggests that brain penetration may involve an active transport phenomena[16] as observed with other analogues such as **(+)-3**.[7]

Acknowledgements

We thank S. Baudouin, D. Briet, F. Gass and B. Martin for technical assistance.

References and Notes

1. For a recent review, see: Doble, A.; *Pharmacol. Ther*. **1999**, *81*, 163, and references cited therein.
2. Choi, D. W. *Neuron,* **1988**, *1*, 623. Sheardown, M. J.; Nielsen, E. O.; Hansen, A. J.; Jacobsen, P.; Honore, T. *Science,* **1990**, *247*, 571. Gill, R.; Brazell, C.; Woodruff, G. N.; Kemp, J. A. *Br. J. Pharmacol.,* **1991**, *103*, 2030. Weiss, J. H.; Koh, J.-Y.; Baimbridge, K. G.; Choi, D. W. *In vitro. Neurology,* **1990**, *40*, 1288. Klockgether, T.; Turski, L.; Honore, T.; Zhang, Z.; Gash, D. M.; Kurlan, R.; Greenmyre, J. T. *Ann. Neurol.,* **1991**, *30,* 717. Lipton, S. A.; Rosenberg, P. A. *N. Engl. J. Med.,* **1994**, *330*, 613. Chapman, A. G.; Smith, S. E.; Meldrum, B. S. *Epilepsy Res.,* **1991**, *9,* 92, and references cited therein.
3. For a recent review, see: Chimirri, A.; Gitto, R.; Zappalà, M. *Exp. Opin. Ther. Patents* **1999**, *9*, 557, and references cited therein.
4. Turski, L.; Huth, A.; Sheardown, M.; McDonald, F.; Neuhaus, R.; Schneider, H. H.; Dirnagl, U.; Wiegand, F.; Jacobsen, P.; Ottow, E. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 10960.
5. Mignani, S.; Aloup, J.-C.; Blanchard, J.-C.; Böhme, G. A.; Boireau, A.; Damour, D.; Debono, M.-W.; Dubroeucq, M.-C.; Genevois-Borella, A.; Imperato, A.; Jimonet, P.; Pratt, J.; Randle, J. C. R.; Reibaud, M.; Ribeill, Y.; Stutzmann, J.-M. *Drug Dev. Res*. **1999**, *48*, 121.
6. Jimonet, P.; Boireau, A.; Chevé, M.; Damour, D.; Genevois-Borella, A.; Imperato, A.; Pratt, J.; Randle, J. C. R.; Ribeill, Y.; Stutzmann, J-M.; Mignani, S. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 2921.
7. Mignani, S.; Bohme, A.; Boireau, A.; Cheve, M.; Damour, D.; Debono, M-W.; Genevois-Borella, A.; Imperato, A.; Jimonet, P.; Pratt, J.; Randle, J. C. R.; Ribeill, Y.; Vuilhorgne, M., Stutzmann, J.-M. *Bioorg. Med. Chem. Lett*. **2000**, accepted for publication.
8. (a) Aloup, J.-C.; Audiau, F.; Barreau, M.; Damour, D.; Jimonet, P.; Genevois-Borella, A.; Jimonet, P.; Mignani, S.; Ribeill, Y. *Patent Applications* WO 96/02544, *Chem. Abstr.* 125, 10817. (b) All compounds described herein gave satisfactory spectroscopic and elemental analysis data. As an example, we report below a full description of what was obtained for compound **4e**: ^{1}H NMR (250 MHz, DMSO, $\delta_{DMSO} = 2.54$ ppm) δ 2.7, (d, $J = 4$, 3H, NCH_3); 4 (s, 2H, H_{10}); 6.1 (bq, NH); 7.34 (dd, $J = 8$ Hz and 1.5, H_7); 7.72 (d, $J = 8$ Hz, H_6) 7.8 (d, $J = 1.5$ Hz, H_9); 8.48 (s, H_1); 8.7 (bs, NH); 12.45 (bs, NH pyrazine). MS (FAB, Gly + SGly) *m/z* 340, MH^+. IR (KBr) cm^{-1}: 3370 (NH), 3200–2250 (OH acid),1712, 1675, 1645 (carbonyl). Elemental analysis: $C_{16}H_{13}N_5O_4$, calcd% C 56.64, H 3.86, N 20.64, O 18.86; found% C 56.70, H 3.60, N 20.61, O 18.92. Precise ^{1}H attributions were obtained via 1D NOE experiments. Strong NOE enhancements were observed between H_{10} and H_1, H_9 in one hand; H_6 and NH (pyrazine); H_7,H_9 and NH (ureido) on the other, thus confirming the skeletal arrangement of these heterocyclic-fused indeno derivatives.
9. (a) Brown, T.; Shaw, G.; Durant, G. J. *J. Chem. Soc. Perkin Trans 1,* **1983**, 809; (b) Branco, P. S.; Prabhakar, S.; Lobo, A. M.; Williams, D. *Tetrahedron,* **1992**, *48*, 6335.
10. Pale yellow oil, $R_f = 0.85$ (silica gel, AcOEt:cycohexane 8:2); ^{1}H NMR (250 MHz, DMSO, $\delta_{DMSO} = 2.54$ ppm) δ 1.3 (t, 3H, CH_3); 1.54 (s, 9H, *tert*-Bu); 4.35 (s, 2H, CH_2O); 7 .85 (s, 1H, H_5); 13.8 (bs, 1H, NH).
11. Honoré T., Drejer J., *J. Neurochem.,* **1988**, *51*, 457.
12. Canton, T.; Doble, A.; Miquet, J.-M.; Jimonet, P.; Blanchard, J.-C. *J. Pharm. Pharmacol*. **1992**, *44*, 812.
13. Swinyard, E. A.; Brown, W. C.; Goodmann, L. S. *J. Pharmacol. Exp. Ther*. **1952**, *106*, 319.
14. Chapman, A. G.; Meldrum, B. S.; Nanji, N.; Watkins, J. C. *Eur. J. Pharmacol*. **1987**, *139*, 91.
15. Debono, M. W.; Le Guern, J.; Canton, T.; Doble, A.; Pradier, L. *Eur. J. Pharmacol*. **1993**, *235*, 289.
16. Auberson, Y. P.; Acklin, P.; Bischoff, S.; Moretti, R.; Ofner, S.; Schmutz, M.; Veenstra, S. J. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 249.