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Preclinical pharmacology of F-98214-TA, a novel potent serotonin and norepinephrine uptake inhibitor with antidepressant and anxiolytic properties

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Abstract *Rationale:* Serotonin (5-HT) and norepinephrine (NE) re-uptake inhibitors (SNRIs) have been proposed to have a higher efficacy and/or faster onset of action than previously available antidepressants. *Objectives:* We examined in biochemical, electrophysiological and behavioural assays the antidepressant properties of (S)-(-)-4-[(3-fluorophenoxy)-phenyl]methyl-piperidine (F-98214-TA), a compound that displays very high affinity for 5-HT and NE transporters. *Results:* F-98214-TA potently inhibited the uptake of both 5-HT and NE into rat brain synaptosomes (IC_{50} =1.9 and 11.2 nM, respectively) and decreased the electrical activity of dorsal raphe serotonergic neurones (ED_{50} =530.3 μ g/kg i.v.), an effect completely abolished by the 5-HT_{1A} antagonist WAY100,635. In acute behavioural assays in mice, the orally administered compound potentiated the 5-hydroxy-tryptophan (5-HTP)-induced syndrome [minimal effective dose (MED)=10 mg/kg], antagonized the hypothermia induced by a high dose of apomorphine (ED_{50} =2 mg/kg) and reduced the immobility in the tail suspension test (MED=10 mg/kg). Moreover, it also decreased

the immobility in the forced swimming test in mice and rats (30 mg/kg, p.o.). Chronic administration of F-98214-TA (14 days, 30 mg kg⁻¹ day⁻¹, p.o.) attenuated the hyperactivity induced by olfactory bulbectomy in rats, confirming its antidepressant-like properties. Interestingly, the same dosage regimen significantly increased the social interaction time in rats, suggesting an additional potential anxiolytic activity. In most assays the compound was more potent than fluoxetine, venlafaxine and desipramine. *Conclusions:* F-98214-TA is a novel SNRI that displays greater potency than other reference antidepressants in animal models predictive of antidepressant and anxiolytic activities.

Keywords Serotonin · Norepinephrine · Uptake inhibitor · Antidepressant · Anxiolytic · Animal model

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Introduction

Depression is a recurrent and life-threatening mental illness with a significant incidence in the population, representing a major social and economic burden (Wong and Licinio 2001). Whereas several classes of antidepressant medications are currently prescribed to treat depression, serious drawbacks exist that require improvement such as the limitations in efficacy, the multiple unwanted side-effects and the slow onset of the therapeutic response. Therefore, the development of novel antidepressants with a significant therapeutic advantage has become a primary objective of pharmaceutical research (Tran et al. 2003).

Although the monoamine deficiency hypothesis has proven to be an excessively simplistic model of the complex pathophysiology of depression (Hindmarch 2001), it has dominated most of the pharmacological approaches to its management. The original hypothesis proposed that depression is caused by a deficiency of brain monoamines, particularly serotonin (5-HT) and norepinephrine (NE), and that antidepressants exerted their effect by increasing the corticolimbic availability of monoamines. A major in-

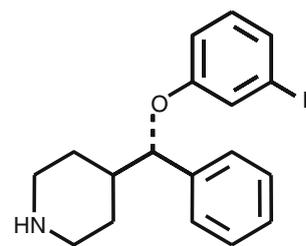
consistency in this hypothesis is the temporal discrepancy between the timing of the primary biochemical effect of drugs (minutes, hours) and the onset of therapeutic action (weeks). More recent theories, based on long-term adaptive changes in the properties of monoaminergic receptors and/or signal transduction mechanisms, have been suggested to explain the delayed therapeutic response to antidepressants (Millan 2004).

Almost all currently marketed antidepressants inhibit monoamine re-uptake, or the degradative enzyme monoamine oxidase, leading to an increase in the availability of 5-HT and/or NE in the synaptic cleft. Tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) interact with the serotonergic and noradrenergic systems but have many adverse effects as a result of direct or indirect interactions with multiple neurotransmitter receptors. The introduction of more selective agents, particularly the selective serotonin re-uptake inhibitors (SSRIs), represented a significant improvement in terms of safety and tolerability, and antidepressants such as fluoxetine, sertraline and paroxetine have become the mainstay drug treatments for depression in the last 20 years (Kerrigan 1998). Nevertheless, they are not completely devoid of side-effects such as nausea, sexual dysfunction and sleep disorders (Goldstein and Goodnick 1998), and they do not offer advantages over older antidepressants in terms of efficacy and faster onset of action (Goodnick and Goldstein 1998).

Clinical studies support that a combined 5-HT and NE enhancement has greater therapeutic efficacy compared with the enhancement of either neurotransmitter alone. Thus, the TCA chlorimipramine, which blocks both NE and 5-HT transporters (SERTs), was shown to have a slightly greater clinical efficacy than the SSRIs (Anderson 1998). Moreover, the enhanced antidepressant activity of the combination of SSRIs and NE-selective TCAs observed in previous studies (Weilburg et al. 1989; Nelson et al. 1991) was confirmed by a recent double blind study combining fluoxetine and desipramine (Nelson et al. 2004).

Based on this rationale, several compounds that inhibit selectively the re-uptake of both 5-HT and NE, and without a significant affinity for neurotransmitter receptors, have been developed for the treatment of depression. These compounds are referred to as mixed 5-HT and NE re-uptake inhibitors (SNRIs), and include venlafaxine, milnacipran and duloxetine. Clinical studies have shown that SNRIs are effective and well-tolerated antidepressants (for reviews see Tran et al. 2003; Zajecka and Albano 2004). Interestingly, meta-analysis of clinical trials suggests that venlafaxine may have somewhat superior response and remission rates than TCAs and SSRIs (Thase et al. 2001; Anderson 2001). In addition, venlafaxine may present efficacy in major depression resistant to SSRIs (Saiz-Ruiz et al. 2002) and a rapid onset of clinical effects when used in high dosages (Montgomery 1995; Entsuah et al. 1998; Burnett and Dinan 1998). There are data suggesting that milnacipran and duloxetine share with venlafaxine an efficacy advantage over SSRIs (Clerc 2001; Goldstein et al.

Fig. 1 Chemical structure of F-98214-TA



F-98214-TA

2004), but the evidence in favour of an early onset of therapeutic response is still awaited.

Although belonging to the same group of antidepressants, SNRIs display some differences in their pharmacological profile that may be relevant to their individual clinical activity. In fact, venlafaxine has a substantially higher affinity for the SERT than for the NE transporter (NET; Bymaster et al. 2001), which correlates with its higher serotonergic activity in vivo (Koch et al. 2003). On the other hand, milnacipran seems to display a limited serotonergic activity (Bel and Artigas 1999; Koch et al. 2003), whereas the recently launched duloxetine is probably the most potent and balanced dual transporter inhibitor (Bymaster et al. 2001). Regarding side-effects, venlafaxine and duloxetine have been associated with a risk of induction of hypertension (Thase 1998; Zajecka and Albano 2004), and milnacipran has a higher prevalence of headache, dry mouth and dysuria compared with the SSRIs (Tran et al. 2003).

With all of the above considerations in mind, the development of new SNRIs may provide the opportunity to obtain compounds with a more suitable blockade of the 5-HT and NE re-uptake processes that, in turn, may result in improved clinical outcomes in depressed patients. As a result of our research programme, the compound (S)-(-)-4-[(3-fluorophenoxy)-phenyl]methyl-piperidine (F-98214-TA) (Fig. 1) was selected from a series of 4-[(aryl)(aryloxy)methyl]-piperidines based on its high affinity for the 5-HT and NETs (Orjales et al. 2003). In the present study, biochemical, electrophysiological and behavioural effects of F-98214-TA are described.

Material and methods

Animals

Unless otherwise specified below, male Wistar rats weighing 200–275 g (FAES FARMA, S.A., Spain), Swiss CD-1 mice weighing 24–35 g (FAES FARMA) and guinea-pigs of 300–350 g body weight (Harlan-Interfauna Ibérica, S.L., Spain) were used. Indicated weights are those upon commencement of experiments. The animals were housed in sawdust-lined cages and in temperature- and humidity-controlled facilities (temperature, 22±1°C; humidity, 65±15%) and were maintained in a 12-h light/dark cycle (lights

on at 0800 hours). Guinea-pigs (four per cage) were adapted to laboratory conditions for at least 2 weeks prior to testing. Rats (six per cage) and mice (ten per cage) were adapted for at least 5 days before the start of experiments. Behavioural assays were carried out in temperature- and humidity-controlled and sound-attenuated rooms. Access to standard chow and water was unrestricted except in acute *in vivo* assays using the oral route of administration. In these cases the animals were submitted to food restriction during 16–18 h before testing to avoid food interference with drug absorption, and water was substituted by a solution containing 8% sucrose and 0.2% NaCl.

All animal studies were performed in accordance with the “European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes” (European Union Directive #86/606/EEC) and the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health.

Binding to monoamine transporters and to neurotransmitter brain receptors

The affinity of F-98214-TA and reference antidepressant agents for SERTs, NETs and dopamine (DA) transporters (DAT), and for diverse brain receptors, was evaluated by using widely described standard procedures summarized in Table 1. Animals were killed by guillotine decapitation, and the whole brains were quickly removed. The various areas were dissected, weighed and immediately frozen at -70°C until use.

Monoamine uptake assays

Crude synaptosomal preparations

Rats were killed by guillotine decapitation, and their brains were quickly removed. The frontal cortex and the striatum

were immediately dissected out, weighed and homogenized in 10 vol of ice-cold 0.32 M sucrose solution with ten passes of a Teflon pestle Potter-S homogenizer (Braun Biotech International, Melsungen, Germany) set at 800 rpm. The homogenates were centrifuged at 1,500 g for 10 min at 4°C , and the resulting supernatants were centrifuged at 18,000 g for 10 min at 4°C . The final pellets were suspended in appropriate volumes of the Krebs bicarbonate physiological solution (pH 7.4) previously bubbled for 10 min with 95% O_2 and 5% CO_2 . To eliminate the endogenous monoamines, the synaptosomal suspensions were incubated at 37°C for 10–15 min. The composition of Krebs bicarbonate medium was 120.8 mM NaCl, 5.9 mM KCl, 2.2 mM CaCl_2 , 1.2 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.2 mM NaH_2PO_4 , 15.5 mM NaHCO_3 and 11.5 mM α -D-glucose. The synaptosomal preparations were immediately used for uptake measurements.

Determination of [^3H]5-HT, [^3H]NE and [^3H]DA uptake

The uptake assay buffer was the Krebs bicarbonate solution containing 25 mM HEPES, 10 μM pargyline, 10 μM reserpine and 0.1% ascorbic acid (pH 7.4). The uptake was performed in triplicate in a final volume of 0.5 ml. In the case of [^3H]5-HT, synaptosomes from the frontal cortex were incubated with the drug concentration and 20 nM [^3H]5-HT (PerkinElmer Life Sciences, 27.5 Ci/mmol) for 2 min at 37°C . Non-specific uptake was determined with 10 μM fluoxetine. For determination of [^3H]NE uptake, synaptosomes from the total cortex were incubated with the drug concentration and 10 nM [^3H]NE (Amersham Pharmacia Biotech, 52 Ci/mmol) for 5 min at 37°C . Non-specific uptake was determined with 10 μM nisoxetine. In DA uptake assays, synaptosomes from striatum were incubated with the drug concentration and 3 nM [^3H]DA (PerkinElmer, 31.6 Ci/mmol) for 5 min at 37°C . Non-specific uptake was determined with 10 μM mazindol.

Table 1 Standard procedures for monoamine transporter and receptor-binding assays

| Site | Specie (tissue) | [^3H]-Ligand (nM) | Non-specific ligand (μM) | Reference |
|--------------------|------------------|------------------------------|---------------------------------------|--------------------------|
| SERT | Rat (TCx) | Paroxetine (0.2) | Fluoxetine (10) | Orjales et al. (2003) |
| NET | Rat (TCx) | Nisoxetine (0.75) | Mazindol (10) | Orjales et al. (2003) |
| DAT | Rat (Str) | WIN-35428 (0.6) | Mazindol (10) | Orjales et al. (2003) |
| 5-HT _{1A} | Rat (TCx) | 8-OH-DPAT (0.4) | 5-HT (10) | Orjales et al. (2003) |
| 5-HT _{2A} | Rat (PFCx) | Ketanserin (0.8) | Methysergide (1) | Orjales et al. (2003) |
| 5-HT _{1B} | Rat (FCx) | GR125743 (2) | 5-HT (10) | Domenech et al. (1997) |
| 5-HT ₃ | Rat (ECx) | LY278584 (1.8) | 5-HT (10) | Orjales et al. (2000) |
| 5-HT ₄ | Guinea pig (Str) | GR113808 (0.15) | 5-HT (100) | Orjales et al. (2000) |
| D ₁ | Rat (Str) | SCH23390 (0.4) | R(+)-SCH23390 (3) | Hess et al. (1986) |
| D ₂ | Rat (Str) | Raclopride (1.0) | (+)-Butaclamol (1) | Orjales et al. (2000) |
| α_1 | Rat (TCx) | Prazosin (0.15) | Phentolamine (10) | Oshita et al. (1991) |
| α_2 | Rat (TCx) | RX821002 (0.75) | Phentolamine (10) | Erdbrügger et al. (1995) |
| H ₁ | Guinea pig (Cb) | Pyrilamine (1.0) | Astemizole (10) | Benavides et al. (1995) |
| Mus | Rat (TCx) | QNB (0.2) | Atropine (10) | Watson et al. (1996) |

TCx Total cortex, Str striatum, PFCx prefrontal cortex, FCx frontal cortex, ECx entorhinal cortex, Cb cerebellum

Data analysis for binding and uptake studies

At the end of the incubation period, the binding reaction or uptake process was stopped, and membranes were filtered through Whatman GF/B filters (Merck 1821.915, Spain) pre-soaked in the respective binding or uptake buffer containing 0.05–0.1% polyethyleneimine, using a Brandel M-24R cell harvester (Reactiva, Barcelona, Spain). Filters were immediately rinsed three times with 4 ml ice-cold buffer or 154 mM NaCl solution, dried and immersed into polyethylene scintillation vials containing 5 ml of Ecoscint-H scintillation cocktail (Itisa Biomédica S.A., Madrid, Spain). The filter-retained radioactivity was determined by scintillation counting. Binding isotherms and data from uptake studies were analysed by nonlinear regression of concentration–effect curve (GraphPad Prism, version 2.0; GraphPad Software Inc., San Diego, CA, USA) to determine IC_{50} values. For each assayed drug the IC_{50} mean value was obtained from a minimum of three independent experiments using 8–14 drug concentrations. For binding studies, the inhibition constants (K_i) were calculated from the Cheng–Prusoff equation: $K_i = IC_{50} / [(L/K_D) + 1]$ (Cheng and Prusoff 1973), where L is the concentration of radiolabelled ligand, and K_D , its dissociation constant determined in specific binding experiments.

Electrophysiological studies

Male Wistar rats weighing 240–280 g (Harlan Ibérica), and allowed to acclimate to the new environment for at least 5 days before the beginning of the experiments, were initially anaesthetized with an intraperitoneal (i.p.) injection of chloral hydrate (400 mg/kg, 5 ml/kg) and maintained under full general anaesthesia with supplementary intravenous (i.v.) doses. The jugular vein was cannulated for drug or anaesthesia administration. Rats were mounted in a stereotaxic frame, and the dura and sagittal sinuses were removed. Single-barrelled glass micropipettes filled with 2 M NaCl containing 2% Pontamine Sky Blue (3–8 M Ω impedance in vitro) were implanted in the dorsal raphe nucleus (DRN; ML 0 mm and AP 1 mm to lambda) with the aid of a microdriver. Serotonergic cell firing in DRN was measured using standard extracellular recording methods (Aghajanian 1978). 5-HT neurones, encountered over 5.6 mm DV, were identified according to their electrophysiological characteristics: a wide-duration action potential (0.8–1.2 ms) and a spontaneous regular pattern of firing (0.5–2.5 Hz). Signals were amplified and filtered, and the integrated firing rates were computed and stored in 10-s bins (Spike2, Cambridge Electronic Design, Cambridge, UK). The mean baseline firing rate was determined for at least 5 min before the administration of cumulative i.v. doses of fluoxetine, venlafaxine and F-98214-TA. The effect of each dose was recorded for 2 min, and a test dose of the 5-HT_{1A} antagonist *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY100,635, 200 μ g/kg i.v.) was given after maximal inhibition of the neuronal firing

to confirm the pharmacological specificity of the response. The number of action potentials after drug administration were quantified (spikes/10 s) and expressed as a percentage of the pre-drug baseline value. Only one neurone per animal was studied. The ED_{50} values were calculated using the programme GraphPad Prism, version 2.0 (GraphPad Software).

Behavioural assays

Potentiation of 5-HTP-induced symptoms in mice

The method was based on that described by Buus Lassen (1978). Mice were orally dosed with the compounds 60 min before administration of 5-HTP (75 mg/kg i.p.). This dose of 5-HTP by itself causes no clear behavioural effects, but produces abnormal aimless jerky hypermotility, head twitches and tremor in mice previously treated with a compound that increases central 5-HT availability. Once the 5-HTP was administered, each mouse was immediately placed singly in a compartment of the Digiscan Activity System (see [Locomotor activity assays in mice and rats](#) below) and behaviour, measured as stereotypy counts, was registered from 15 to 45 min after 5-HTP injection.

Apomorphine-induced hypothermia in mice

The procedure was adapted from that described by Fuxe and Sjöqvist (1972). Animals were orally dosed with the compounds, and after a period of 20 min, a first rectal temperature was measured using a digital thermometer (mod. 0331, Panlab sl, Barcelona, Spain). After 10 min, apomorphine was injected (16 mg/kg s.c.), and 20 min after the injection of apomorphine, climbing behaviour was assessed in a wire-mesh cylinder (10 cm diameter, 12 cm high) according to the following rating scale: 0, all four paws on the floor; 1, one or two paws on the cylinder; and 2, three or four paws in the cylinder. A second rectal temperature was measured 10 min later. The difference ($^{\circ}$ C) between the two temperature values was calculated for each animal.

Tail suspension test in mice

Male NMRI mice weighing 25–34 g (Charles River, Barcelona, Spain) were used. The procedure was adapted from that described by Steru et al. (1985). In dose-response assays, 60 min after oral administration of the compounds, each mouse was suspended by its tail from a hook connected to a force transducer [F 30 type 372, Hugo Sachs Elektronik (HSE), Germany], which allowed the detection of all movements of the animal by connecting it to a polygraph recorder (WR 3101, HSE). The duration of immobility (s) was recorded over a 6-min testing period. In time-course assays independent groups of animals were tested 1, 3, 6, 18 and 24 h after the oral administration of

compounds (30 mg/kg). The percentage of reduction of the immobility time was obtained for each treatment group.

Forced swimming test in mice

Male NMRI mice weighing 25–34 g (Charles River) were used. The procedure was based on that described by Porsolt et al. (1978a). Sixty minutes after the oral administration of compounds, mice were individually forced to swim in an open cylindrical container (diameter 18 cm, height 27 cm), containing 17 cm of water at $22\pm 1^\circ\text{C}$. The total duration of immobility during the last 4 min of a single 6-min test session was scored. The animal was judged to be immobile when it ceased struggling or swimming and remained floating, making only the necessary movements to keep its head above the water. Data were analysed using the Kruskal–Wallis test followed by the Mann–Whitney *U* test. The percentage of reduction of the immobility time was obtained for each treatment group.

FST in rats

The test was performed as previously described (Porsolt et al. 1978b). Rats were individually forced to swim for 15 min in an open cylindrical container (diameter 22 cm, height 36 cm) containing 22 cm of water at $24\pm 1^\circ\text{C}$ (pretest session). Animal weights were in the range of 120–150 g to prevent an animal from touching the bottom of the container with their tail. Once the pretest session had finished, rats were removed and allowed to dry for 15 min in a cage placed 30 cm below an infrared lamp before returning to their home cage. Twenty-four hours later, they were placed again in the container for 5 min (test session), and the total duration of immobility was recorded. A rat was judged to be immobile when it ceased struggling and remained floating, making only the necessary movements to keep its head above the water. Drugs were administered orally three times: 23.5, 5 and 1 h before the test session.

Olfactory bulbectomy in rats

The method was based on that described by Cairncross et al. (1978). Sprague–Dawley rats (Centre d'Élevage R. Janvier, France) were anaesthetized with 2,2,2-tribromoethanol (250 mg/kg i.p. 5 ml/kg). A mid-line scalp incision was made, and bilateral 2-mm diameter burr holes were drilled through the skull and dura mater, 6 mm anterior to bregma and 2 mm lateral to the mid-line. The olfactory bulbs were removed using a water suction pump. The holes in the skull were packed with homeostatic sponge, and the incision was closed with wound clips. Sham-operated rats received burr holes only. The animals were given 14 days to recover following surgery and were handled daily to eliminate any aggressiveness that may otherwise arise. Imipramine (32 mg/kg) and F-98214-TA (10 and 30 mg/kg) were orally administered once daily in the morning (be-

tween 9:00 am and 12:00 nn) from days 14 to 27 post-surgery. On the morning of day 28 (22–26 h after the last administration), rats were placed into a highly illuminated circular arena (90 cm diameter) surrounded by an aluminium wall. The arena floor was divided into 10-cm squares, and ambulation (number of squares crossed) was measured for each animal during a 3-min period by an observer blind to treatments. At the end of the experiments, the rats were killed, and their brains were inspected. In the case of damage to the cortex or olfactory bulbs being not completely removed, animals were excluded from the analysis of the data.

Social interaction test in rats

The procedure was adapted from that of File et al. (1996). In acute assays, animals were established four rats per cage for at least 14 days before the experiment was carried out. On the testing day, animals received an i.p. injection with test compounds, and 30 min after, weight-matched pairs (± 10 g) of rats, having received the same treatment and belonging to different cages, were placed in opposite corners of a highly illuminated (300 lux) open-topped arena (62×62×42 cm) for a 7.5-min session. A camera was mounted about 2 m above the arena, and the camera was connected to a monitor in an adjacent room. The observer recorded from the screen the duration of active social interaction (i.e. the time spent in grooming, following, sniffing, biting, jumping or crawling over or under the other animal). If animals remained adjacent to each other without any movement for longer than 5 s, scoring was stopped until active social interaction resumed. At the end of each session, the test arena was carefully cleaned.

In chronic assays, rats (four per cage) were orally administered during 14 days with vehicle, imipramine (32 mg/kg), venlafaxine (30 mg/kg) or F-98214-TA (30 mg/kg). On day 15, 22–24 h after the last administration, each animal was firstly individually tested during 30 min for locomotor activity in the Digiscan Activity System (see [Locomotor activity assays in mice and rats](#) below) and was returned to its home cage. After at least 1 h had passed, pairs of rats, having received the same treatment, were tested for social interaction as described for acute experiments.

Locomotor activity assays in mice and rats

The testing apparatus consisted of eight Digiscan activity monitors connected to two Digiscan analyzers (Omnitech Electronics Inc., Columbus, OH, USA). Monitors detect interruptions of 16 photo beams spaced 2.5 cm apart and 2.5 cm above the floor. For spontaneous locomotor activity experiments, rats (one animal per activity monitor) and mice (two animals per activity monitor) were orally treated with drugs and immediately placed in individual test chambers. Distance travelled (cm) was recorded during 3 h (three intervals, 60 min each).

Statistical analysis

Results of the FST and social interaction assays in rats, 5-HTP potentiation and locomotor activity studies were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test to detect a significant difference from the control group. A non-parametric test was used when normality and/or equal variance test failed, that is, in the tail suspension and forced swimming tests (FSTs) in mice, as well as in the olfactory bulbectomy model, where the Kruskal–Wallis test was carried out, followed by the Mann–Whitney *U* test, to compare treatment groups from the vehicle-treated group. In the apomorphine-induced hypothermia assay, the percentage of reduction in the hypothermic effect of apomorphine was calculated for each treatment group, and ED₅₀ values plus 95% confidence limits (CL) were obtained according to the method of Litchfield and Wilcoxon (1949). Analyses were conducted using the SigmaStat programme for IBM computers (2.03, SPSS Inc., Chicago, IL, USA) with the exception of the Mann–Whitney *U* test, which was performed using the Analyse-it/Excel programme (version 1.67).

Drugs

F-98214-TA and WAY100,635 HCl were internally synthesized, and venlafaxine HCl was extracted from Vandral 75 (A. Toledo). Fluoxetine HCl was obtained from Interquímica SA (Barcelona, Spain), and mazindol, nisoxetine·HCl, 5-hydroxy-DL-tryptophan, apomorphine HCl, desipramine HCl and imipramine HCl were purchased from Sigma (Madrid, Spain). For binding assays, drugs were dissolved in dimethylsulfoxide (10⁻² M), and dilutions were made in the buffer as appropriate. In electrophysiological studies fluoxetine and venlafaxine were dissolved in saline, whereas F-98214-TA was dissolved in pure ethanol, making dilutions in saline as appropriate to a final concentration of 5% ethanol. For behavioural studies drugs were dissolved or suspended in saline with a drop of Tween 80 and administered in a constant volume of 10 ml/kg. Doses of F-98214-TA and venlafaxine are expressed as the bases.

Results

Affinity of F-98214-TA and reference antidepressants for cerebral rat monoamine transporters and for neurotransmitter brain receptors

F-98214-TA exhibited a marked affinity for SERT and NET in the rat cerebral cortex, with a slight preference for the former (K_i in nM, 2.0±0.4 and 13.5±0.5, respectively). A similar pattern of dual interaction with SERT and NET was obtained with venlafaxine. However, its affinities at these sites were 26- and 50-fold lower, respectively, than those of F-98214-TA (Table 2). Fluoxetine displayed a pronounced affinity for SERT (K_i =30.9 nM) and a negligible

Table 2 Affinities of F-98214-TA and reference antidepressants for monoamine transporters and for brain receptors related to monoaminergic transmission or to adverse effects

| Site | F-98214-TA | Fluoxetine | Venlafaxine | Desipramine |
|--------------------|------------|------------|-------------|-------------|
| SERT | 2.0±0.4 | 30.9±7.7 | 52.0±8.5 | 342±120 |
| NET | 13.5±0.5 | >1,000 | 719±61 | 2.7±0.3 |
| DAT | 680±168 | >1,000 | >1,000 | >1,000 |
| 5-HT _{1A} | >1,000 | >1,000 | >1,000 | >1,000 |
| 5-HT _{1B} | >1,000 | >1,000 | >1,000 | >1,000 |
| 5-HT _{2A} | >1,000 | >1,000 | >1,000 | ~1,000 |
| 5-HT ₃ | >1,000 | >1,000 | Nd | >1,000 |
| 5-HT ₄ | >1,000 | >1,000 | >1,000 | >1,000 |
| α ₁ | >1,000 | >1,000 | >1,000 | 127±9.6 |
| α ₂ | >1,000 | >1,000 | >1,000 | >1,000 |
| D ₁ | >1,000 | >1,000 | Nd | >1,000 |
| D ₂ | >1,000 | >1,000 | >1,000 | >1,000 |
| Mus | >1,000 | >1,000 | >1,000 | 262±13.9 |
| H ₁ | >1,000 | >1,000 | >1,000 | 140±8.5 |

Data are expressed as mean±SEM K_i (nM) of at least three separate experiments, each performed in triplicate
Nd Not determined

affinity for NET, whereas desipramine showed an opposite pattern of preference (K_i =2.7 nM for NET). All compounds, including F-98214-TA, displayed a low affinity for DAT. The affinity of the compound, as well as that of fluoxetine and venlafaxine for several brain receptors, including monoaminergic, muscarinic and histaminergic receptors, was negligible (K_i >1,000 nM, Table 2), whereas desipramine displayed a moderate affinity for α₁-adrenergic, H₁-histamine and muscarinic receptors.

Effect of F-98214-TA as compared to reference antidepressants on the [³H]monoamine uptake into rat brain synaptosomes

Consistently with its high affinity for SERT and NET, F-98214-TA potently inhibited the uptake of both [³H]5-HT and [³H]NE into rat cerebral synaptosomes (IC₅₀=1.9±2.4 and 11.2±4.8 nM, respectively, Table 3). Venlafaxine inhibited also the uptake of both [³H]5-HT and [³H]NE, although with clearly less potency than F-98214-TA. As expected, fluoxetine selectively inhibited the synaptosomal uptake of [³H]5-HT, while desipramine inhibited potently and selectively the [³H]NE uptake. Unlike reference an-

Table 3 Influence of F-98214-TA and reference antidepressants on [³H]monoamine uptake into rat cerebral synaptosomes

| | F-98214-TA | Fluoxetine | Venlafaxine | Desipramine |
|-----------------------|------------|------------|-------------|-------------|
| [³ H]5-HT | 1.9±2.4 | 59.9±14.9 | 116.7±19.0 | >1,000 |
| [³ H]NE | 11.2±4.8 | >1,000 | 253±61 | 19.2±3.6 |
| [³ H]DA | 86.8±33.2 | >1,000 | >1,000 | >1,000 |

Data are presented as mean±SEM IC₅₀ (nM) of at least three independent experiments, each performed in triplicate

tidepressants, F-98214-TA displayed a moderate potency to inhibit the [3 H]DA uptake ($K_i=86.8$ nM).

Effect of F-98214-TA, fluoxetine and venlafaxine on the electrical activity of serotonergic neurones in DRN

Single-unit recordings showed the ability of F-98214-TA to dose dependently and completely inhibit the firing rate of serotonergic neurones localized into the DRN ($ED_{50}=530.3\pm 47.0$ μ g/kg i.v., Fig. 2). This action was completely abolished by the selective 5-HT $_{1A}$ antagonist WAY 100,635 (200 μ g/kg i.v., data not shown). Fluoxetine and venlafaxine displayed a similar pattern of inhibition, with respective ED_{50} values of 787.0 ± 104.2 and 358.7 ± 44.8 μ g/kg i.v.

Potentiation of 5-HTP-induced symptoms in mice

F-98214-TA dose-dependently increased the stereotypy counts induced by 5-HTP in mice with a minimal effective dose (MED) of 10 mg/kg (Fig. 3 and Table 4). In a similar way, venlafaxine and (with less efficacy) fluoxetine significantly potentiated the syndrome induced by the 5-HT precursor at the dose of 30 mg/kg. Desipramine did not show any 5-HTP potentiating effect.

Apomorphine-induced hypothermia in mice

Administration of apomorphine induced a reduction in core temperature of mice in the order of 4°C, and this effect was potently antagonized by F-98214-TA in a dose-dependent manner ($ED_{50}=2$ mg/kg, Fig. 4 and Table 4). Desipramine was slightly more potent, and venlafaxine was slightly less potent than F-98214-TA, whereas fluoxetine modestly

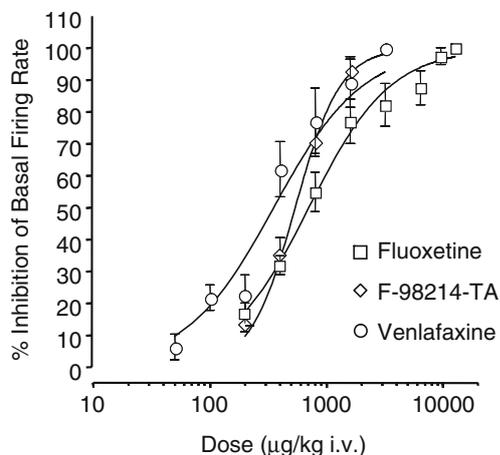


Fig. 2 Dose-dependent effect of cumulative doses of F-98214-TA, venlafaxine and fluoxetine on the firing of DRN serotonergic neurones after intravenous (i.v.) injection. Data are expressed as the percentage of inhibition of the basal electrical activity. Fluoxetine and F-98214-TA, $n=8$ rats/group; venlafaxine, $n=5$ rats/group

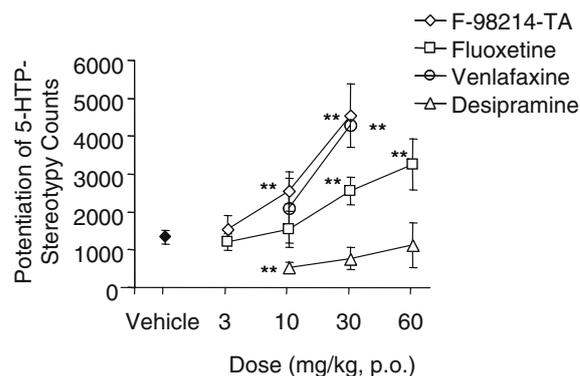


Fig. 3 Potentiation of 5-HTP-induced symptoms in mice by F-98214-TA and reference antidepressants. Drugs were administered 60 min before 5-HTP (75 mg/kg i.p.). Stereotypy counts were measured from 15 to 45 min after injection of 5-HTP. Data represent mean \pm SEM ($n\geq 9$). **indicates $p < 0.01$ vs vehicle-treated group (Dunnett's test following ANOVA)

attenuated the apomorphine-induced hypothermia, reaching a percentage of 60 % of antagonism at the dose of 90 mg/kg. None of the compounds affected core temperature alone or climbing behaviour induced by apomorphine (not shown).

Tail suspension test in mice

In dose-response assays in the tail suspension test, F-98214-TA induced at the dose of 10 mg/kg (p.o.) a significant and marked reduction (73%) of mice immobility (Fig. 5a and Table 4). Venlafaxine and desipramine mimicked these actions but were less potent, reaching statistical significance at the dose of 30 mg/kg. Only high doses of fluoxetine (90 mg/kg) displayed activity in this model.

In the time-course study (Fig. 5b) the anti-immobility effect of F-98214-TA (30 mg/kg) was present 18 h after administration, whereas that of venlafaxine was significant only 1 h after administration. In an additional experiment, mice were given vehicle or F-98214-TA (30 mg/kg, p.o.), and 18 h later, the mice were singly placed in the locomotor activity apparatus, and the distance travelled was recorded for a 30-min period (three samples, 10 min each). No effect of F-98214-TA on motor activity was detected at any interval (total distance travelled in control animals, $2,368\pm 689$ cm; F-98214-TA-treated group, $2,197\pm 131$ cm; $n=16$).

FST in mice and rats

Oral administration of F-98214-TA significantly reduced the immobility time in the FST in mice (Fig. 6a and Table 4). The reduction of the immobility time was about 80% at 30 mg/kg. In a similar way, venlafaxine dose-dependently reduced the immobility of the animals with a percentage of reduction of the immobility of 48% at the same dose. High doses of desipramine (60 mg/kg) and fluoxetine (90 mg/kg) were required to reach statistical

Table 4 Summary of actions of F-98214-TA and reference antidepressants in behavioural models of potential antidepressant activity and on locomotor activity in rodents

| Drug | 5-HTP | APO | TST ^a | FST mice ^a | FST rats ^a | OB | Locomotor activity ^b | |
|-------------|-------|---------------------------|------------------|-----------------------|-----------------------|-----|---------------------------------|-------------|
| | MED | ED ₅₀ (95% CL) | MED | MED | MED | MED | Mice | Rats |
| F-98214-TA | 10 | 2.0 (1.0–3.9) | 10 (71%) | 30 (80%) | 30 (67%) | 30 | 60↓ | NE (10–120) |
| Fluoxetine | 30 | 60.7 (24.9–148) | 90 (45%) | 90 (37%) | NE (30–120) | – | NE (30–90) | NE (30–60) |
| Venlafaxine | 30 | 3.6 (1.8–7.4) | 30 (71%) | 30 (47%) | 60 (54%) | >30 | NE (10–60) | 60↑ |
| Desipramine | NE | 0.7 (0.3–1.8) | 30 (60%) | 60 (54%) | 30 (48%) | – | 60↓ | 30↓ |

Doses are expressed in mg/kg p.o.

5-HTP Potentiation of 5-HTP symptoms in mice, APO antagonism of apomorphine-induced hypothermia in mice, TST tail suspension test in mice, OB olfactory bulbectomy model in rats, NE no effect (in parenthesis, evaluated dose range), ↓ decrease, ↑ increase

^aIn parenthesis, percentage of immobility reduction

^bdoses inducing significant changes are indicated

significance. In the FST in rats, F-98214-TA significantly decreased the duration of the immobility when administered at 30 mg/kg (Fig. 6b and Table 4). Venlafaxine and desipramine also reduced immobility time at doses of 60 and 30 mg/kg, respectively, whereas fluoxetine was inactive even at the dose of 120 mg/kg.

Olfactory bulbectomy in rats

Removal of the olfactory bulbs produced a characteristic hyperactivity in bulbectomized animals when placed in the open field as compared to sham-operated animals (Fig. 7). This hyperactivity was significantly reduced by chronic treatment with imipramine (32 mg/kg) and F-98214-TA (30 mg/kg). Both compounds induced significant increases in ambulation in sham-operated animals.

Social interaction test in rats

Acute injection of imipramine (3 mg/kg), venlafaxine (10 mg/kg) and F-98214-TA (10 mg/kg) induced a sig-

nificant decrease in the time spent in social interaction by pairs of unfamiliar rats placed in a novel and highly illuminated environment without a concomitant effect in locomotion (Table 5). Imipramine at the dose of 10 mg/kg produced a reduction in both social interaction time and locomotor activity of the animals. Repeated treatment with F-98214-TA (30 mg/kg p.o.) for 14 days induced a significant increase in active social interaction among pairs of rats, whereas imipramine and venlafaxine (32 and 30 mg/kg p.o., respectively) induced a non-significant increase in social interaction time (Table 5). F-98214-TA

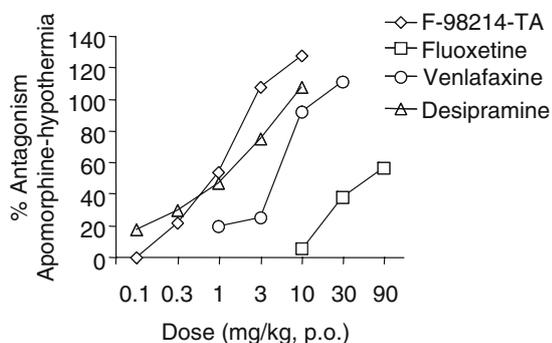


Fig. 4 Antagonism of apomorphine-induced hypothermia in mice by F-98214-TA and reference antidepressants. Compounds were administered p.o. 30 min before apomorphine injection (16 mg/kg s.c.). Hypothermia was estimated by measuring the rectal temperature 10 min before and 30 min after apomorphine treatment. Data are expressed as the percentage reversal of each treatment group of the hypothermic effect induced by apomorphine ($n=10$). The maximal decrease reached in a vehicle-treated group was 4.8°C, with absolute core temperatures of 37.1±0.2°C (before apomorphine) and 32.3±0.2°C (30 min after apomorphine injection)

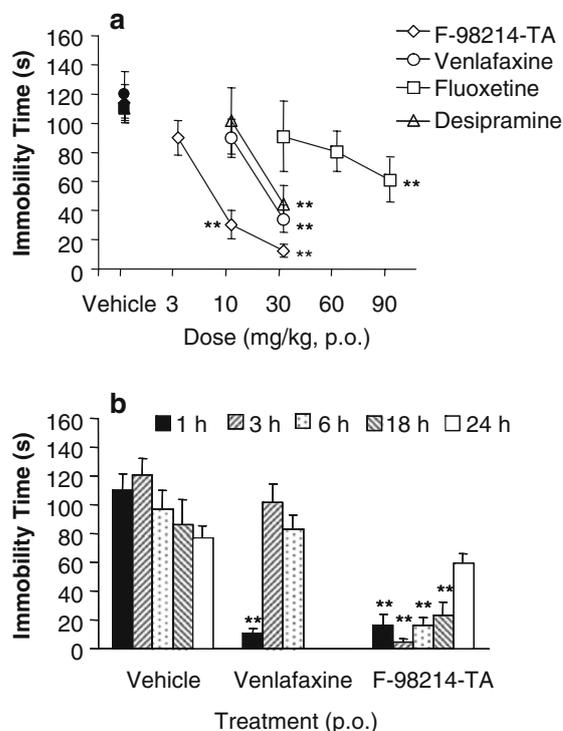


Fig. 5 Effect of F-98214-TA and reference antidepressants upon the duration of immobility in the tail suspension test in mice. **a** Dose-response assays: drugs were administered p.o. 60 min before test (6 min). Vehicle groups: □ indicates F-98214-TA; •, venlafaxine; ▽, fluoxetine and desipramine. **b** Time-course assays: immobility was evaluated in independent groups 1, 3, 6, 18 and 24 h after the administration of compounds. Data represent mean±SEM ($n\geq 9$). ** indicates $p<0.01$ vs respective vehicle-treated group (Kruskal–Wallis followed by Mann–Whitney U test)

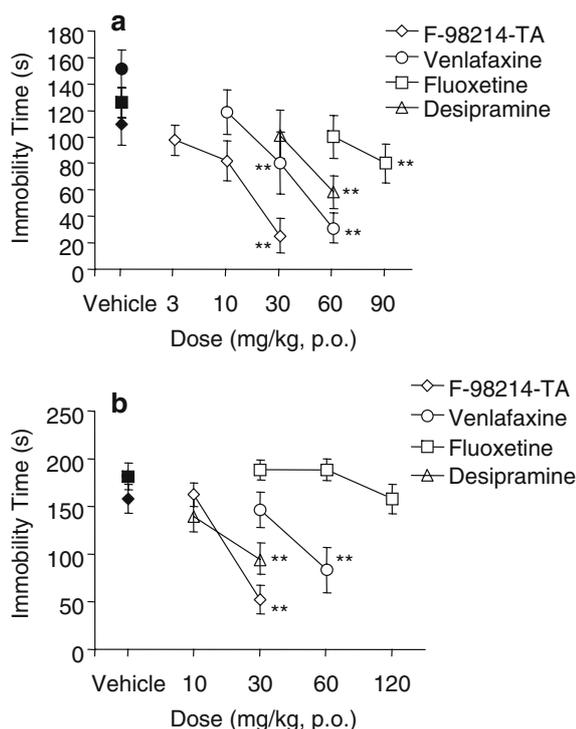


Fig. 6 Effect of F-98214-TA and reference antidepressants upon the duration of immobility in the FST in mice (a) and rats (b). **a** Compounds were administered, and 60 min later, immobility time was evaluated during the last 4 min of a 6-min test session. Vehicle-treated groups: □ indicates F-98214-TA; •, venlafaxine; and ◻, fluoxetine and desipramine. **b** Animals were submitted to a first swimming session (15 min), and 24 h later, a second test session (5 min) was carried out. Compounds were administered 23.5, 5 and 1 h before the test session. Vehicle-treated groups: □ indicates F-98214-TA; and ◻, venlafaxine, fluoxetine and desipramine. Statistical analysis was performed using means of Kruskal–Wallis followed by a Mann–Whitney *U* test (a) and ANOVA followed by Dunnett's test (b). Data represent mean±SEM ($n \geq 9$). ** indicates $p < 0.01$ vs respective vehicle-treated group

and venlafaxine chronically treated animals showed no changes in the distance travelled, whereas imipramine produced a significant reduction in this parameter.

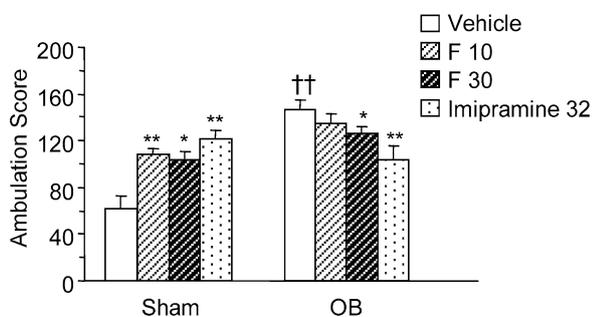


Fig. 7 Effect of 14 days of treatment with F-98214-TA (F) and imipramine on the olfactory bulbectomy-induced hyperactivity in the open field. Doses are expressed in $\text{mg kg}^{-1} \text{ day}^{-1}$ (p.o.). Data represent mean±SEM ($n \geq 8$). †† indicates $p < 0.01$ vehicle-treated olfactory bulbectomized (OB) rats vs. sham-operated controls (Mann–Whitney *U* test). * indicates $p < 0.05$ and **, $p < 0.01$ vs. respective vehicle-treated group (Kruskal–Wallis followed by Mann–Whitney *U* test)

Spontaneous locomotor activity in mice and rats

Significant reductions in spontaneous locomotor activity in mice were observed 1 h after the oral administration of F-98214-TA, and desipramine at the dose of 60 mg/kg (Tables 4 and 6). None of the evaluated compounds significantly affected locomotor activity in mice 3 h after drug administration.

In rats neither fluoxetine (30 and 60 mg/kg) nor F-98214-TA (10–120 mg/kg) induced significant changes in spontaneous locomotor activity. Desipramine (30 and 60 mg/kg) and venlafaxine (60 mg/kg) induced, respectively, a significant decrease and increase in the distance-travelled parameter 3 h after their administration (Tables 4 and 6).

Discussion

The results obtained in the present work indicate that F-98214-TA is a potent SNRI, active in rodent models predictive of antidepressant and anxiolytic activity, and displays a higher potency than the reference antidepressants in most of the performed assays.

In vitro binding experiments showed that the affinity of F-98214-TA for rat-brain SERT is clearly higher than those of the SSRI fluoxetine, the SNRI venlafaxine and the tricyclic desipramine. It also possesses a high affinity for NET, only surpassed by desipramine, whereas its affinity for DAT is low, reflecting the high degree of selectivity of F-98214-TA for SERT and NET. The affinity profiles of the comparative antidepressants for the aminergic transporters are in line with those previously described (Owens et al. 1997; Muth et al. 1991).

In correlation with its affinities for the transporters, synaptosomal uptake studies showed that F-98214-TA inhibited 5-HT re-uptake site with 30- and 60-fold higher potencies than fluoxetine and venlafaxine, respectively, whereas the inhibition potency at the NE re-uptake site was similar to that of desipramine and 23-fold higher than that of venlafaxine. Regarding the dopaminergic system, a poor correlation was found between the (low) affinity of F-98214-TA for DAT and its moderate potency to block the re-uptake process. However, such potency resulted to be 40-fold and eightfold lower than that for inhibiting 5-HT and NE uptake. The relevance of this dopaminergic component is unclear at present, taking into account that no elevation in locomotor activity was observed in rats, a response expected in the case of an increased dopaminergic neurotransmission (Bymaster et al. 2003). Interestingly, F-98214-TA did not reveal any significant affinity for neurotransmitter receptors involved in the mediation of cardiovascular, sedative and anticholinergic side-effects typically associated with TCAs (i.e. adrenergic- α_1 , histamine- H_1 and muscarinic receptors, Owens et al. 1997; Brunello and Racagni 1998).

The suppression of the firing rate of serotonergic neurons by re-uptake inhibitors could be considered as an in vivo index of 5-HT re-uptake blocking potency (Béique

Table 5 Effect of acute and repeated administration of imipramine, venlafaxine and F-98214-TA in the social interaction test and locomotor activity in rats

| | Acute (i.p.) | | | Chronic (p.o.) | | |
|-------------|--------------|-------------|------------|--|-------------|-------------|
| | Dose mg/kg | SI time (s) | DT (cm) | Dose mg kg ⁻¹ day ⁻¹ | SI time (s) | DT (cm) |
| Imipramine | 0 | 133.3±7.6 | 3,364±449 | 0 | 125.4±14.0 | 4,118±308 |
| | 3 | 104.0±8.4* | 2,673±253 | 32 | 152.1±8.0 | 2,468±304** |
| | 10 | 92.9±5.3** | 1,474±228* | | | |
| Venlafaxine | 0 | 128.4±7.2 | 3,364±449 | 0 | 87.6±6.5 | 2,592±226 |
| | 3 | 111.4±5.3 | 3,056±436 | 30 | 112.6±12.3 | 3,073±301 |
| | 10 | 97.9±7.9* | 2,235±308 | | | |
| F-98214-TA | 0 | 108.3±9.7 | 3,364±449 | 0 | 125.4±14.0 | 4,118±308 |
| | 3 | 83.3±8.1 | 2,659±324 | 30 | 164.9±8.1* | 3,533±357 |
| | 10 | 79.5±6.3* | 2,146±287 | | | |

Data represent mean±SEM. Locomotor activity was evaluated as distance travelled (DT) during 30 min using the Digiscan system. Social interaction (SI time, s) was evaluated in pairs of rats during 7.5 min under high light/unfamiliar conditions. In acute studies, tests were performed 30 min after i.p. injection, and independent groups were used for locomotor activity ($n=9-10$) and social interaction test ($n=8-10$ pairs of rats). In chronic assays, drugs were given once daily during 14 days, and locomotor activity ($n=14-20$) and social interaction time ($n=7-10$) were evaluated 22–25 h after the last administration
* $p<0.05$, ** $p<0.01$ vs. vehicle-treated group (Dunnett's test following ANOVA)

et al. 1999). In our studies, the electrical activity of serotonergic neurones in the DRN was similarly suppressed by venlafaxine, fluoxetine and F-98214-TA, and this effect was totally reversed by WAY100,635, indicating the involvement of 5-HT_{1A} auto-receptors in this effect (Bjorvatn et al. 2000). Venlafaxine and fluoxetine data are in good agreement with those previously reported by others (Muth et al. 1991; Czachura and Rasmussen 2000). However, it is noteworthy that venlafaxine, which displayed low potency in inhibiting the synaptosomal uptake of [³H]5-HT, showed a relative high potency in suppressing the firing rate of raphe neurones. This discrepancy between the in vivo and in vitro activities has been previously described not only for venlafaxine (Béique et al. 1999) but also for the compound S33005 (Millan et al. 2001a). In addition to pharmacokinetic factors, it has been suggested that a particularly high activity of re-uptake inhibitors in vivo may reflect the existence of specific cerebral isoforms of

monoamine transporters, for which these agents display a higher affinity than revealed by in vitro studies (Millan et al. 2001a). Additional studies are needed to further clarify this issue.

The serotonergic and noradrenergic properties of F-98214-TA were confirmed by in vivo studies in mice. The behavioural tests used in the present study, the potentiation of 5-HTP-induced behaviour and the antagonism of apomorphine-induced hypothermia, have previously been related to serotonergic and noradrenergic mechanisms, respectively (Buus Lassen 1978; Stenger et al. 1987; Redrobe et al. 1998). In agreement with 5-HT uptake data, F-98214-TA was the most potent in the 5-HTP model (MED 10 mg/kg), whereas a threefold higher dose of fluoxetine and venlafaxine was required to significantly enhance the effect of 5-HTP. Desipramine did not potentiate 5-HTP-induced responses but was the most potent drug in antagonizing the hypothermic effect induced by

Table 6 Effect of fluoxetine, desipramine, venlafaxine and F-98214-TA on spontaneous locomotor activity in mice and rats

| | Dose (mg/kg p.o.) | Locomotor activity, mice | | Locomotor activity, rats | |
|-------------|-------------------|--------------------------|-----------|--------------------------|-------------|
| | | 1 h | 3 h | 1 h | 3 h |
| Vehicle | – | 3,021±154 | 5,936±472 | 3,350±508 | 6,292±780 |
| Desipramine | 10 | 2,503±343 | 3,932±538 | – | – |
| | 30 | 2,265±255 | 4,098±528 | 2,215±234 | 2,978±368* |
| | 60 | 1,636±341* | 4,005±855 | 2,127±246 | 2,955±375* |
| Fluoxetine | 30 | 2,807±273 | 5,256±584 | 2,518±250 | 3,260±321 |
| | 60 | 2,680±243 | 4,847±707 | 2,380±251 | 4,299±594 |
| | 90 | 2,909±302 | 5,161±676 | – | – |
| Vehicle | – | 2,755±176 | 5,825±591 | 3,578±343 | 5,112±497 |
| Venlafaxine | 10 | 2,418±189 | 5,054±511 | – | – |
| | 30 | 2,610±241 | 5,237±530 | 3,085±333 | 5,458±741 |
| | 60 | 2,350±214 | 5,520±474 | 4,223±503 | 9,210±1394* |
| F-98214-TA | 10 | 2,258±258 | 5,096±842 | 3,589±675 | 4,467±647 |
| | 30 | 2,060±278 | 5,921±701 | 3,149±361 | 4,284±706 |
| | 60 | 1,490±145* | 6,440±952 | 3,011±326 | 4,058±333 |
| | 120 | – | – | 2,830±291 | 3,888±496 |

Results are expressed as mean ±SEM ($n\geq 9$) of distance travelled (cm). Animals were placed in the activity metre immediately after administration
* $p<0.01$ vs respective control group (Dunnett's test following ANOVA)

apomorphine. F-98214-TA also potently blocked the apomorphine hypothermic effect, whereas fluoxetine was nearly inactive. The relative high potency of venlafaxine in this model contrasted with its preferential interaction with SERT vs NET *in vitro*. Similarly, *in vivo* effects of venlafaxine on electrical activity of noradrenergic neurones in the locus coeruleus, as well as on extracellular levels of NE in the frontal cortex, also reflect a pronounced influence upon noradrenergic transmission (Millan et al. 2001a). Moreover, electrophysiological data have previously shown that noradrenergic effects of venlafaxine are probably not mediated by serotonergic mechanisms (Béique et al. 1999). As mentioned above, the peculiarly high *in vivo* potency of venlafaxine may reflect the interaction with distinct NE re-uptake processes, which can be distinguished by their different sensitivities to antidepressants (Hughes and Stanford 1998).

In spite of differences between laboratories, predictive assays of antidepressant activity such as the tail suspension test and the FST in mice are responsive to major classes of antidepressants, including tricyclics, SSRIs and SNRIs (Dalvi and Lucki 1999; Redrobe et al. 1998; Millan et al. 2001b). In agreement with previous reports (Steru et al. 1985), the tail suspension test was, in our hands, more sensitive than the FST to the action of antidepressant drugs. F-98214-TA induced marked antidepressant-like effects and was the most potent drug in both behavioural models. The weak antidepressant effect displayed by fluoxetine in both tests was not surprising, since similar results, or even inactivity, have been previously described with this drug (Sanchez and Meier 1997; Nielsen et al. 2004). In this regard, the selection of the mouse strain and the mechanism of action of the drug seem to be crucial factors in the detection of the antidepressant-like effect in these tests (David et al. 2003; Ripoll et al. 2003). In accordance with other authors (Van der Heyden et al. 1987), previous internal data from our laboratory (not shown) suggested that the NMRI strain of mice was the most suitable for the tail suspension test, and the use of the same strain of mice in the two models was preferred.

An important factor frequently associated with drug therapy adherence is the frequency of medication doses. In this regard, it is generally accepted that treatment regimens that require once-daily dosing may result in better patient's compliance than those requiring multiple doses per day. SSRIs usually need to be taken once a day, whereas the standard formulations of SNRIs are generally given twice a day. In this regard, it is noteworthy that the antidepressant-like activity of F-98214-TA in the mouse tail suspension test was present 18 h after its acute administration. This could suggest a better kinetic profile when compared to venlafaxine which, according to its short half-life (Burnett and Dinan 1998), was active only 1 h after its acute administration. Pharmacokinetic assays are being carried out to confirm this potential benefit.

In the FST in rats, F-98214-TA and desipramine exhibited a more potent antidepressant-like effect than venlafaxine. Conflicting results were obtained regarding the effect of SSRIs in this model, since fluoxetine resulted

inactive in the tested dose range (30–120 mg/kg), whereas sertraline (30 mg/kg, data not shown) induced a marked antidepressant-like effect. It has been argued that the efficiency of SSRIs in the rat FST is highly dependent on the experimental procedure and animal strain (Borsini and Meli 1988; Rénéric and Lucki 1998). Thus, whereas some researchers have found a clear anti-immobility effect with these compounds (Cervo et al. 1991; Overstreet 1993), others describe only a weak activity, generally at high doses, or even inactivity (Lucki et al. 1994; Borsini 1995; Mochizuki et al. 2002). The re-evaluation of SSRIs in a modified FST that provides separate scores for two active behaviours (swimming and climbing) resulted in a consistent antidepressant-like effect characterized by a selective increase of swimming (Detke et al. 1995). The present data using the traditional version of the model agree with those of Mochizuki et al. (2002), who reported conflicting results regarding the effects of SSRIs in the rat FST and suggested the apparent relevance of the noradrenergic system in this test. In this regard, the potency exhibited by F-98214-TA is remarkable.

The olfactory bulbectomy in rats has been validated as a model of depression, and has the advantage over most other current models, in that many of the behavioural changes induced by olfactory bulbectomy are reversed by chronic but not acute antidepressant treatment (Kelly et al. 1997; Cryan et al. 1998). In a similar way to that observed with other antidepressants, including SNRIs (McGrath and Norman 1998; Redmond et al. 1999), chronic treatment with F-98214-TA (30 mg kg⁻¹ day⁻¹ p.o.) significantly reduced the increased locomotor activity seen in the control bulbectomized rats. This finding strongly supports the clinical potential of F-98214-TA as an antidepressant.

An intriguing finding in the olfactory bulbectomy study was the increase of ambulation in sham-operated animals after repeated treatment with imipramine and F-98214-TA. A possible stimulant effect can be ruled out to explain this action, since neither imipramine nor F-98214-TA enhanced the spontaneous locomotor activity of rats after repeated treatment. On the other hand, the exposure of animals to stressful conditions, by placing them in a highly illuminated, open and novel arena, makes the open field a suitable procedure to measure anxiety-like behaviours. Therefore, we hypothesized that the observed hyperactive behaviour in the open field in imipramine- and F-98214-TA-treated sham-operated animals may be reflecting a decrease in the stress-induced inhibition of exploratory behaviour. To test this possibility, the effect of imipramine and F-98214-TA was evaluated in the social interaction test, a well-established model of anxiety that mimicks the aversive conditions of the open field. Since no data are available concerning the effect of chronic administration of SNRIs in this model, we also analyzed the effects of venlafaxine in the social interaction test. As reported for some SSRIs (Borsini et al. 2002), acute injection of venlafaxine, F-98214-TA and the lowest dose of imipramine reduced social interaction without any significant alteration of motor functions, a response which may be interpreted as

a specific enhancement in anxiety. A possible mechanism that may account for this effect is the indirect activation of 5-HT_{2C} receptors, as it has been previously suggested to explain the acute anxiogenic effect of SSRIs (Dekeyne et al. 2000). The decrease in social interaction elicited by the highest dose of imipramine may be reflecting the sedative properties of this agent. In contrast to the acute actions, the sedative effect of imipramine after chronic treatment was accompanied by a marked, but not significant, increase in the social interaction time. These results suggest that adaptive changes after prolonged imipramine treatment may recruit anxiolytic mechanisms that blunt and override its acute anxiogenic effect and are in line with the anxiolytic-like activity reported for imipramine by Popik and Vetulani (1993) under similar conditions. Nevertheless, since the apparent anxiolytic effect of imipramine could be masked by the marked sedative effect, evaluation of lower doses with less or no effects on spontaneous locomotor activity may help to demonstrate more clearly this activity.

In addition to their efficacy in the treatment of depression, newer antidepressants, particularly SSRIs and venlafaxine, are increasingly used as first-line agents in the treatment of anxiety disorders (Kent et al. 1998; Rickels et al. 2000). Interestingly, 14 days of F-98214-TA treatment produced a significant increase in rat social interaction with no concurrent effect on locomotor activity consistent with anxiolysis. The magnitude of this increase was similar to that observed in rats treated with chlordiazepoxide for 5 days (5 mg kg⁻¹ day⁻¹ i.p., data not shown). Although chronic treatment with antidepressants is rather inactive in this test (Borsini et al. 2002), our results are in line with the robust anxiolytic-like effect reported with paroxetine after 3, but not 2, weeks of treatment (Duxon et al. 2000). If, as it has been argued by these authors, the social interaction test models the temporal effects of SSRIs in the clinic, the present data suggest that F-98214-TA may have a faster onset of anxiolytic-like action than paroxetine. Nevertheless, it is important to note that, although similar high light/unfamiliar conditions were used in both studies, some methodological issues, such as the time interval between the last dose of the drug and the test (1 h vs about 24 h in our study), can explain this different onset of action. Finally, the same dose of venlafaxine (30 mg/kg) also increased social behaviour in the absence of motor effects, but this change was not statistically significant. A higher dose or a longer treatment period may be required to induce a clear anxiolytic-like effect.

It is noteworthy that active doses of F-98214-TA in behavioural tests (10 and 30 mg/kg) had no effect on spontaneous locomotor activity in rodents. Moreover, the transient sedation observed in mice at the dose of 60 mg/kg and the lack of any significant effect of the compound on spontaneous locomotor activity in rats in the whole dose range examined (10–120 mg/kg) may be reflecting a substantial therapeutic window to a generalized disruption of behaviour. Venlafaxine was devoid of any effect on spontaneous locomotor activity in mice but induced a locomotor stimulant effect in rats at the dose of 60 mg/kg.

These findings are consistent with other works showing species differences in the effects of venlafaxine on locomotor activity (Rogóz et al. 1998; Brocco et al. 2002). In mice, previous data have reported no changes (Rogóz et al. 1998) or increases in locomotor activity (Redrobe et al. 1998; Brocco et al. 2002), although this latter effect has been suggested to reflect not an effect upon motor function but upon arousal (Brocco et al. 2002). On the other hand, our data reveal an acute stimulant action of venlafaxine in the rat, which contrast with the sedative properties described by other authors in this specie (Rogóz et al. 1998; Rénéric and Lucki 1998; Brocco et al. 2002; Millan et al. 2001b). However, it is important to note that such effect was observed only during the 3-h testing period, a rather long period not examined in the abovesited studies, and which could account for the discrepant results. The current results are in line with recent observations reporting increases in both locomotor activity and striatal DA levels after chronic treatment with venlafaxine in female rats (de Oliveira et al. 2004). In addition, as the stimulant effect of venlafaxine may have contributed to its antidepressant-like effect in the rat FST, additional spontaneous locomotor activity studies would be helpful to rule out this possibility. Finally, fluoxetine did not produce any significant influence on spontaneous locomotor activity in mice and rats, whereas desipramine, as expected from its antihistaminergic properties, induced a significant reduction in locomotor activity in both species.

In conclusion, F-98214-TA is a potent SNRI with antidepressant-like activity, following acute and repeated administration in rodents and without significant affinity for receptors that could mediate adverse effects. Interestingly, the compound is more potent than desipramine, fluoxetine and venlafaxine in the majority of the experimental tests performed in this study. Our data also suggest that this drug could induce a prolonged therapeutic effect. The potential anxiolytic activity of F-98214-TA after chronic administration represents an added benefit, having in mind the overlap between anxiety and depression disorders. Upcoming clinical studies will soon indicate how the preclinical antidepressant-like efficacy translates into antidepressant activity in humans.

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References

- Aghajanian GK (1978) Feedback regulation of central monoaminergic neurons: evidence from single cell recording studies. In: Youdin MBH, Lovenberg W, Sharman DF, Lagnado JR (eds) *Essays in neurochemistry and neuropharmacology*. Wiley, New York, pp 1–32

- Anderson IM (1998) SSRIs versus tricyclic antidepressants in depressed outpatients: a meta-analysis of efficacy and tolerability. *Depress Anxiety* 7:11–17
- Anderson IM (2001) Meta-analytical studies on new antidepressants. *Br Med Bull* 57:161–178
- Béique JC, De Montigny C, Blier P, Debonnel G (1999) Venlafaxine: discrepancy between in vivo 5-HT and NE reuptake blockade and affinity for reuptake sites. *Synapse* 32:198–211
- Bel N, Artigas F (1999) Modulation of the extracellular 5-hydroxytryptamine brain concentrations by the serotonin and noradrenaline reuptake inhibitor, milnacipran. *Neuropsychopharmacology* 21:745–754
- Benavides J, Schoemaker C, Dana Y, Claustre M, Delahaye M, Prouteau P, Manoury P, Allen J, Scatton B, Langer SZ, Arbilla S (1995) In vivo and in vitro interaction of the novel selective histamine H₁ receptor antagonist Mizolastine with H₁ receptors in the rodent. *Arzneim Forsch* 45:551–558
- Bjorvatn B, Fornal CA, Martín FJ, Metzler CW, Jacobs BL (2000) Venlafaxine and its interaction with WAY100,635: effects on serotonergic unit activity and behaviour in cats. *Eur J Pharmacol* 404:121–132
- Borsini F (1995) Role of the serotonergic system in the forced swimming test. *Neurosci Biobehav Rev* 19:377–395
- Borsini F, Meli A (1988) Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology* 94:147–160
- Borsini F, Podhorna J, Marazziti D (2002) Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology* 163:121–141
- Brocco M, Dekeyne A, Veiga S, Girardon S, Millan MJ (2002) Induction of hyperlocomotion in mice exposed to a novel environment by inhibition of serotonin reuptake. A pharmacological characterization of diverse classes of antidepressant agents. *Pharmacol Biochem Behav* 71:667–680
- Brunello N, Racagni G (1998) Rationale for the development of noradrenaline reuptake inhibitors. *Hum Psychopharmacol* 13:S13–S19
- Burnett FE, Dinan TG (1998) Venlafaxine. Pharmacology and therapeutic potential in the treatment of depression. *Hum Psychopharmacol* 13:153–162
- Buus Lassen J (1978) Potent and long-lasting potentiation of two 5-hydroxytryptophan-induced effects in mice by three selective 5-HT uptake inhibitors. *Eur J Pharmacol* 47:351–358
- Bymaster FP, Dreshfield-Ahmad LJ, Threlkeld PG, Shaw JL, Thompson L, Nelson DL, Hemrick-Luecke SK, Wong DT (2001) Comparative affinity of duloxetine and venlafaxine for serotonin and norepinephrine transporters in vitro and in vivo, human serotonin receptor subtypes, and other neuronal receptors. *Neuropsychopharmacology* 25:871–880
- Bymaster FP, McNamara RK, Tran PV (2003) New approaches to developing antidepressants by enhancing monoaminergic neurotransmission. *Expert Opin Investig Drugs* 12:531–543
- Cairncross KD, Cox B, Forster C, Wren AF (1978) A new model for the detection of antidepressant drugs: olfactory bulbectomy in the rat compared with existing models. *J Pharmacol Methods* 1:131–143
- Cervo L, Grignaschi G, Rossi C, Samanin R (1991) Role of serotonergic neurons in the effect of sertraline in rats in the forced swimming test. *Eur J Pharmacol* 196:217–222
- Cheng YC, Prusoff WH (1973) Relationship between inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I₅₀) of an enzymatic reaction. *Biochem Pharmacol* 22:3099–3108
- Clerc G, Milnacipran/Fluvoxamine Study Group (2001) Antidepressant efficacy and tolerability of milnacipran, a dual serotonin and noradrenaline reuptake inhibitor: a comparison with fluvoxamine. *Int Clin Psychopharmacol* 16:145–151
- Cryan JF, Mc Grath C, Leonard BE, Norman TR (1998) Combining pindolol and paroxetine in an animal model of chronic antidepressant action. Can early onset of action be detected? *Eur J Pharmacol* 352:23–28
- Czachura JF, Rasmussen K (2000) Effects of acute and chronic administration of fluoxetine on the activity of serotonergic neurons in the dorsal raphe nucleus of the rat. *Naunyn-Schmiedeberg's Arch Pharmacol* 362:266–275
- Dalvi A, Lucki I (1999) Murine models of depression. *Psychopharmacology* 147:14–16
- David DJP, Renard CE, Jolliet P, Hascoët M, Bourin M (2003) Antidepressant-like effects in various mice strains in the forced swimming test. *Psychopharmacology* 166:373–382
- de Oliveira RA, Cunha GM, Borges KD, de Bruin GS, dos Santos-Filho EA, Viana GS, de Bruin VM (2004) The effect of venlafaxine on behaviour, body weight and striatal monoamine levels on sleep-deprived female rats. *Pharmacol Biochem Behav* 79:499–506
- Dekeyne A, Denorme B, Monneyron S, Millan MJ (2000) Citalopram reduces social interaction in rats by activation of serotonin (5-HT)_{2C} receptors. *Neuropharmacology* 39:1114–1117
- Detke MJ, Rickels M, Lucki I (1995) Active behaviours in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. *Psychopharmacology* 121:66–72
- Domenech T, Beleta J, Palacios JM (1997) Characterization of human serotonin 1D and 1B receptors using [³H]-GR-125743, a novel radiolabelled serotonin 5-HT_{1D/1B} receptor antagonist. *Naunyn-Schmiedeberg's Arch Pharmacol* 356:328–334
- Duxon MS, Starr KR, Upton N (2000) Latency to paroxetine-induced anxiolysis in the rat is reduced by co-administration of the 5-HT_{1A} receptor antagonist WAY100635. *Brit J Pharmacol* 130:1713–1719
- Entsuah R, Derivan A, Kikta D (1998) Early onset of antidepressant action of venlafaxine: pattern analysis in intent-to-treat patients. *Clin Ther* 20:517–526
- Erdbrügger W, Raulf M, Otto T, Michel MC (1995) Does [³H]-2-methoxy-Idazoxan (RX821002) detect more alpha₂-adrenoceptor agonist high affinity sites than [³H]-Rauwolscine? A comparison of nine tissues and cell lines. *J Pharmacol Exp Ther* 273:1287–1294
- File SE, Gonzalez LE, Andrews N (1996) Comparative study of pre- and postsynaptic 5-HT_{1A} receptor modulation of anxiety in two ethological animal tests. *J Neurosci* 16:4810–4815
- Fuxe K, Sjöqvist F (1972) Hypothermic effect of apomorphine in the mouse. *J Pharm Pharmacol* 24:702–705
- Goldstein BJ, Goodnick PJ (1998) Selective serotonin reuptake inhibitors in the treatment of affective disorders-III. Tolerability, safety and pharmacoeconomics. *J Psychopharmacol* 12:S55–S87
- Goldstein DJ, Lu Y, Detke MJ, Wiltse C, Mallinckrodt C, Demitrack MA (2004) Duloxetine in the treatment of depression: a double-blind placebo-controlled comparison with paroxetine. *J Clin Psychopharmacol* 24:389–399
- Goodnick PJ, Goldstein BJ (1998) Selective serotonin reuptake inhibitors in the treatment of affective disorders-II. Efficacy and quality of life. *J Psychopharmacol* 12:S21–S54
- Hess EJ, Battaglia G, Norman AB, Iorio LC, Creese I (1986) Guanine nucleotide regulation of agonist interactions at [³H]-SCH 23390-labeled D₁ dopamine receptors in rat striatum. *Eur J Pharmacol* 121:31–38
- Hindmarch I (2001) Expanding the horizons of depression: beyond the monoamine hypothesis. *Hum Psychopharmacol Clin Exp* 16:203–218
- Hughes ZA, Stanford SC (1998) Evidence from microdialysis and synaptosomal studies of rat cortex for noradrenaline uptake sites with different sensitivities to SSRIs. *Br J Pharmacol* 124:1141–1148
- Kelly JP, Wrynn AS, Leonard BE (1997) The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther* 74:299–316
- Kent JM, Coplan JD, Gorman JM (1998) Clinical utility of the selective reuptake inhibitors in the spectrum of anxiety. *Biol Psychiatry* 44:812–824

- Kerrigan F (1998) Antidepressant patents: 1995–1997. *Expert Opin Ther Pat* 8:439–460
- Koch S, Hemrick-Luecke SK, Thompson LK, Evans DC, Threlkeld PG, Nelson DL, Perry KW, Bymaster FP (2003) Comparison of effects of dual transporter inhibitors on monoamine transporters and extracellular levels in rats. *Neuropharmacology* 45:935–944
- Litchfield JT, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther* 96:99–113
- Lucki I, Singh A, Kreiss DS (1994) Antidepressant-like behavioural effects of serotonin receptor agonists. *Neurosci Biobehav Rev* 18:85–95
- McGrath C, Norman TR (1998) The effect of venlafaxine treatment on the neurochemical changes in the olfactory bulbectomized rat. *Psychopharmacology* 136:394–401
- Millan MJ (2004) The role of monoamines in the actions of established and “novel” antidepressant agents: a critical review. *Eur J Pharmacol* 500:371–384
- Millan MJ, Gobert A, Lejeune F, Newman-Tancredi A, Rivet JM, Auclair A, Peglion JL (2001a) S33005, a novel ligand at both serotonin and norepinephrine transporters: I. Receptor binding, electrophysiological, and neurochemical profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. *J Pharmacol Exp Ther* 298:565–580
- Millan MJ, Dekeyne A, Papp M, La Rochelle CD, MacSweeney C, Peglion JL, Brocco M (2001b) S33005, a novel ligand at both serotonin and norepinephrine transporters: II. behavioural profile in comparison with venlafaxine, reboxetine, citalopram, and clomipramine. *J Pharmacol Exp Ther* 298:581–591
- Mochizuki D, Tsujita R, Yamada S, Kawasaki K, Otsuka Y, Hashimoto S, Hattori T, Kitamura Y, Miki N (2002) Neurochemical and behavioural characterization of milnacipran, a serotonin and noradrenaline reuptake inhibitor in rats. *Psychopharmacology* 162:323–332
- Montgomery SA (1995) Rapid onset of action of venlafaxine. *Int Clin Psychopharmacol* 10:21–27
- Muth EA, Moyer JA, Haskins JT, Andree TH, Husbands GEM (1991) Biochemical, neurophysiological, and behavioural effects of Wy-45,233 and other identified metabolites of the antidepressant venlafaxine. *Drug Dev Res* 23:191–199
- Nelson JC, Mazure CM, Bowers MB, Jatlow PI (1991) A preliminary, open study of the combination of fluoxetine and desipramine for rapid treatment of depression. *Arch Gen Psychiatry* 48:303–307
- Nelson JC, Mazure CM, Jatlow PI, Bowers MB, Price LH (2004) Combining norepinephrine and serotonin reuptake inhibition mechanism for treatment of depression: a double-blind, randomized study. *Biol Psychiatry* 55:296–300
- Nielsen DM, Carey GL, Gold LH (2004) Antidepressant-like activity of corticotropin-releasing factor type-1 receptor antagonists in mice. *Eur J Pharmacol* 499:135–146
- Orjales A, Alonso-Cires L, López-Tudanca PL, Tapia I, Labeaga L, Mosquera R (2000) Synthesis and 5-HT₃ receptor affinity of new quinolinecarboxylic acid derivatives. *Drug Des Discov* 16:271–279
- Orjales A, Mosquera R, Toledo A, Pumar MC, García N, Cortizo L, Labeaga L, Innerarity A (2003) Syntheses and binding studies of new [(aryl)(aryloxy)methyl] piperidine derivatives and related compounds as potential antidepressant drugs with high affinity for serotonin (5-HT) and norepinephrine (NE) transporters. *J Med Chem* 46:5512–5532
- Oshita M, Kigoshi S, Muramatsu I (1991) Three distinct binding sites for [³H]-prazosin in the rat cerebral cortex. *Br J Pharmacol* 104:961–965
- Overstreet DH (1993) The Flinders sensitive line rats: a genetic animal model of depression. *Neurosci Biobehav Rev* 17:51–68
- Owens MJ, Morga WN, Plott SJ, Nemeroff CB (1997) Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. *J Pharmacol Exp Ther* 283:1305–1322
- Popik P, Vetulani J (1993) Similar action of imipramine and arginine-vasopressin in the social interaction test. *Pol J Pharmacol* 45:323–325
- Porsolt R, Bertin A, Jalfre M (1978a) Behavioural despair in rats and mice: strain differences and the effects of imipramine. *Eur J Pharmacol* 51:291–294
- Porsolt R, Anton G, Blavet N, Jalfre M (1978b) Behavioural despair in rats: a new model sensitive to antidepressant treatments. *Eur J Pharmacol* 47:379–391
- Redmond AM, Kelly JP, Leonard BE (1999) The determination of the optimal dose of milnacipran in the olfactory bulbectomized rat model of depression. *Pharmacol Biochem Behav* 62:619–623
- Redrobe JP, Bourin M, Colombel MC, Baker GB (1998) Dose-dependent noradrenergic and serotonergic properties of venlafaxine in animal models indicative of antidepressant activity. *Psychopharmacology* 138:1–8
- Rénéric JP, Lucki I (1998) Antidepressant behavioural effects by dual inhibition of monoamine reuptake in the rat forced swimming test. *Psychopharmacology* 136:190–197
- Rickels K, Pollack MH, Sheehan DV, Haskins JT (2000) Efficacy of extended-release venlafaxine in non-depressed outpatients with generalized anxiety disorder. *Am J Psychiatry* 157:968–974
- Ripoll N, David DJP, Dailly E, Hascoët M, Bourin M (2003) Antidepressant-like effects in various mice strains in the tail suspension test. *Behav Brain Res* 143:193–200
- Rogóż Z, Dziedzicka-Wasylewska M, Maj J (1998) The pharmacological profile of venlafaxine, a new antidepressant, given acutely. *Pol J Pharmacol* 50:107–115
- Saiz-Ruiz J, Ibanez A, Diaz-Marsa M, Arias F, Padin J, Martin-Carrasco M, Montes JM, Ferrando L, Carrasco JL, Martin-Ballesteros E, Jorda L, Chamorro L (2002) Efficacy of venlafaxine in major depression resistant to selective serotonin reuptake inhibitors. *Prog Neuropsychopharmacol Biol Psychiatry* 26:1129–1134
- Sanchez C, Meier E (1997) Behavioural profiles of SSRIs in animal models of depression, anxiety and aggression. Are they all alike? *Psychopharmacology* 129:197–205
- Stenger A, Couzinier J-P, Briley M (1987) Psychopharmacology of midalcipran, 1-phenyl-1-diethyl-amino-carbonyl-2-aminomethylcyclopropane hydrochloride (F 2207), a new potential antidepressant. *Psychopharmacology* 91:147–153
- Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology* 85:367–370
- Thase ME (1998) Effects of venlafaxine on blood pressure: a meta-analysis of original data from 3744 depressed patients. *J Clin Psychiatry* 59:502–508
- Thase ME, Entsuah AR, Rudolph RL (2001) Remission rates during treatment with venlafaxine or selective serotonin reuptake inhibitors. *Br J Psychiatry* 178:234–241
- Tran PV, Bymaster FP, McNamara RK, Potter WZ (2003) Dual monoamine modulation for improved treatment of major depressive disorder. *J Clin Psychopharmacol* 23:78–86
- Van Der Heyden JAM, Molewijk E, Olivier B (1987) Strain differences in response to drugs in the tail suspension test for antidepressant activity. *Psychopharmacology* 92:127–130
- Watson M, Roeske WR, Yamamura HI (1996) [³H]-Pirenzepine and [³H]-Quinuclidinyl benzilate binding to rat cerebral cortical and cardiac muscarinic cholinergic sites: characterization and regulation of antagonist binding to putative muscarinic subtypes. *J Pharmacol Exp Ther* 237:419–427
- Weilburg JB, Rosenbaum JF, Biederman J, Sachs GS, Pollack MH, Kelly K (1989) Fluoxetine added to non-MAOI antidepressants converts nonresponders to responders: a preliminary report. *J Clin Psychiatry* 50:447–449
- Wong ML, Licinio J (2001) Research and treatment approaches to depression. *Nat Rev Neurosci* 2:343–351
- Zajacka JM, Albano D (2004) SNRIs in the management of acute major depressive disorder. *J Clin Psychiatry* 65:11–18