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RESEARCH PAPER

Preclinical characterization of WAY-211612: a dual 5-HT uptake inhibitor and 5-HT$_{1A}$ receptor antagonist and potential novel antidepressant

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Background and purpose: As a combination of 5-HT selective reuptake inhibitor (SSRI) with 5-HT$_{1A}$ receptor antagonism may yield a rapidly acting antidepressant, WAY-211612, a compound with both SSRI and 5-HT$_{1A}$ receptor antagonist activities, was evaluated in preclinical models.
Experimental approach: Occupancy studies confirmed the mechanism of action of WAY-211612, while its *in vivo* profile was characterized in microdialysis and behavioural models.
Key results: WAY-211612 inhibited 5-HT reuptake (K_i = 1.5 nmol·L^{-1}; K_B = 17.7 nmol·L^{-1}) and exhibited full 5-HT$_{1A}$ receptor antagonist activity (K_i = 1.2 nmol·L^{-1}; K_B = 6.3 nmol·L^{-1}; I_{max} 100% in adenyl cyclase assays; K_B = 19.8 nmol·L^{-1}; I_{max} 100% in GTPγS). WAY-211612 (3 and 30 mg·kg^{-1}, po) occupied 5-HT reuptake sites in rat prefrontal cortex (56.6% and 73.6% respectively) and hippocampus (52.2% and 78.5%), and 5-HT$_{1A}$ receptors in the prefrontal cortex (6.7% and 44.7%), hippocampus (8.3% and 48.6%) and dorsal raphe (15% and 83%). Acute or chronic treatment with WAY-211612 (3–30 mg·kg^{-1}, po) raised levels of cortical 5-HT approximately twofold, as also observed with a combination of an SSRI (fluoxetine; 30 mg·kg^{-1}, s.c.) and a 5-HT$_{1A}$ antagonist (WAY-100635; 0.3 mg·kg^{-1}, s.c). WAY-211612 (3.3–30 mg·kg^{-1}, s.c.) decreased aggressive behaviour in the resident-intruder model, while increasing the number of punished crossings (3–30 mg·kg^{-1}, i.p. and 10–56 mg·kg^{-1}, po) in the mouse four-plate model and decreased adjunctive drinking behaviour (56 mg·kg^{-1}, i.p.) in the rat scheduled-induced polydipsia model.
Conclusions and implications: These findings suggest that WAY-211612 may represent a novel antidepressant.
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Keywords: depression; antidepressant; fluoxetine; serotonin; microdialysis; rodent

Abbreviations: DAT, dopamine transporter; NET, noradrenaline transporter; OCD, obsessive compulsive disorder; SERT, 5-HT transporter; SSRIs, 5-HT selective reuptake inhibitors

Introduction

In the late 1980s and early 1990s, the introduction of selective inhibitors of 5-HT uptake (SSRIs), including fluoxetine (Prozac®), paroxetine (Paxil®) and sertraline (Zoloft®), revolutionized the clinical management of depression. Despite their success, a major limitation of SSRIs, and one that extends to all other classes of antidepressants as well, is the 2 to 6 week delay in onset of therapeutic activity. This lengthy time to achieve remission is suspected to result, in large part, from indirect activation of somatodendritic 5-HT$_{1A}$ autoreceptors (Chaput *et al.*, 1986; Invernizzi *et al.*, 1996). Support for this hypothesis stems from research findings showing that acute SSRI administration elevates 5-HT levels in several brain regions including the dorsal raphe nuclei (Gartside *et al.*, 1995). This local elevation in 5-HT engages inhibitory 5-HT$_{1A}$ autoreceptors in the dorsal raphe to inhibit 5-hydroxytryptaminergic cell firing and dampen subsequent 5-HT release in terminal brain areas (Invernizzi *et al.*, 1992; 1996). However, following long-term SSRI administration, these 5-HT$_{1A}$ autoreceptors desensitize, resulting in a pronounced increase in 5-HT levels compared with acute treatment (Kreiss and Lucki, 1995). Therefore, based on this 5-HT$_{1A}$ receptor desensitization hypothesis, a strategy that combines SSRI and 5-HT$_{1A}$ autoreceptor antagonism – the

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latter mechanism mimicking the receptor desensitization that occurs following chronic antidepressant therapy – would be expected to produce a more rapid increase in central 5-HT and potentially yield an antidepressant that shortens the duration of time to reach clinical efficacy (see Artigas *et al*., 1996, Adell *et al*., 2005 and Millan, 2006). Evidence suggests that combining SSRI activity with antagonism of both 5-HT$_{1A}$ and 5-HT$_{1B}$ autoreceptors also elicits efficacy in preclinical models of depression and anxiety (Watson and Dawson, 2007).

The neurochemical hypothesis described above is supported by a plethora of preclinical studies demonstrating that pretreatment with selective 5-HT$_{1A}$ antagonists, such as WAY-100635, augment SSRI-induced changes in cortical 5-HT (Hjorth *et al*., 1997; Sharp *et al*., 1997; Dawson and Nguyen, 1998; Beyer *et al*., 2002), as well as the antidepressant-like effects of SSRIs in the rodent schedule-induced polydipsia (SIP), resident-intruder and social interaction models (Mitchell and Redfern, 1997; Duxon *et al*., 2000; Hogg and Dalvi, 2004). Additionally, clinical data utilizing this combination strategy indicate that the antidepressant activity of SSRIs is accelerated and/or enhanced when combined with the mixed 5-HT$_{1A}$/β adrenoceptor antagonist, pindolol (Blier and Bergeron, 1998; Artigas *et al*., 2006). While these clinical results remain somewhat controversial (see Berman *et al*., 1997), preclinical data obtained by co-administering an SSRI with a selective 5-HT$_{1A}$ antagonist (as cited above) suggest that a single compound combining SSRIs with 5-HT$_{1A}$ antagonism should have a favourable therapeutic utility in the treatment of depression. Given the merits of a single molecule possessing both properties, several groups have attempted to develop such dual-acting compounds (Mewshaw *et al*., 2004; Rocco *et al*., 2004; Evrard *et al*., 2005; Hatzenbuhler *et al*., 2006).

WAY-211612 represents a novel and selective molecule with dual SSRI/5-HT$_{1A}$ receptor antagonist properties. We have illustrated this compound's dual activity *in vitro* and by using *in vivo* receptor/transporter occupancy studies. Moreover, as most hypotheses of depression postulate that medications capable of increasing 5-HT transmission are effective antidepressants (see Blier and de Montigny, 1994), the neurochemical effects of acute and chronic administration of WAY-211612 were studied by *in vivo* microdialysis. The neurochemical effects of WAY-211612 were compared with the combination of fluoxetine and WAY-100635, and all studies were performed in the rat frontal cortex. Additionally, the behavioural effects of WAY-211612 were evaluated in a variety of preclinical models of depression (resident intruder), obsessive compulsive disorder (OCD; SIP) and anxiety (four-plate). Overall, the results from these preclinical experiments suggest that WAY-211612 is an innovative molecule combining 5-HT reuptake inhibition with selective 5-HT$_{1A}$ receptor antagonism that, by virtue of its dual mechanism of action, may represent a potentially novel antidepressant agent.

Methods

Animals

All animal procedures and care complied with the specifications of both the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Wyeth's Institutional Animal Care and Use Committee.

In vitro *pharmacology*

Primary activities. 5-HT transporter (SERT). *Binding affinity:* A protocol similar to that used by Cheetham *et al*. (1993) was used to determine the affinity of compounds for the SERT. Briefly, frontal cortical membranes prepared from male Sprague-Dawley rats were incubated with [^{3}H]-paroxetine (0.1 nmol·L^{-1}) for 60 min at 25°C. All tubes also contained either vehicle, test compound (one to eight concentrations) or a saturating concentration of fluoxetine (10 μmol·L^{-1}) to define non-specific binding. All reactions were terminated by the addition of ice-cold Tris buffer followed by rapid filtration using a Tom Tech® filtration device to separate bound from free ^{3}H-paroxetine. Bound radioactivity was quantified using a Wallac 1205 Beta Plate® counter. Non-linear regression analysis was used to determine IC_{50} values which were converted to Ki values using the method of Cheng and Prusoff (1973); Ki = IC50/[(Radioligand conc.)/(1 + K_D)].

Functional activity: Human JAR cells, natively expressing SERT (see Ramamoorthy *et al*., 1995 for details) were plated at 90 000 cells per well in Falcon Optilux 96-well plates (Cat. No. 353947) in growth medium containing RPMI 1640 (Gibco, Cat. No. 72400), 10% FBS (Irvine, Cat. No. 3000), 1% sodium pyruvate (Gibco, Cat. No. 1136) and 0.25% glucose. At 24 h post-plating, cells were stimulated with 40 nmol·L^{-1} staurosporine to enhance SERT expression. Cells were subsequently returned to incubation at 37°C. At 48 h post-plating, the cell media was removed and replaced by 200 μL Krebs-HEPES assay buffer (25 mmol·L^{-1} HEPES, 120 mmol·L^{-1} NaCl, 5 mmol·L^{-1} KCl, 2.5 mmol·L^{-1} $CaCl_2$, 1.2 mmol·L^{-1} $MgSO_4.7H_2O$, 2 mg·mL^{-1} glucose, 0.2 mg·mL^{-1} ascorbic acid, 1 μmol·L^{-1} pargyline, pH 7.4). All drug treatments were dissolved in an aqueous solution containing 4% DMSO. 25 μL of each drug treatment was added in triplicate to desired wells and incubated for 5 min. Total uptake wells were defined as those wells receiving 25 μL of vehicle and non-specific uptake wells weare defined as those receiving 20 μmol·L^{-1} fluoxetine. Following drug pretreatment, a final concentration of 12 nmol·L^{-1} [^{3}H] 5-HT (Perkin Elmer No. NET-498) was added to each well and the plates incubated at 37°C for an additional 5 min. The uptake reaction was terminated by centrifugation at 1500× *g* for 5 min. The supernatant was subsequently aspirated and the cells washed with 250 μL of ice-cold 50 mmol·L^{-1} Tris-HCl (pH 7.4). The cells were centrifuged for an additional 5 min at 1500× *g*, the supernatant was aspirated and the cells lysed following the addition of 25 μL of 0.25 mol·L^{-1} NaOH. Each well was supplemented with 100 μL of Microscint-20 scintillation cocktail and the plates were sealed. Plates were counted at 1 min per well on a TopCount NXT. Non-linear regression analysis was used to determine IC_{50} values which were converted to K_B values using the method described by Cheng and Prusoff (1973).

5-HT$_{1A}$ receptors. *Binding affinity:* Receptor-binding studies were performed using human 5-HT$_{1A}$ receptors stably expressed in CHO DUK cells. [^{3}H]-8-OH-DPAT-binding studies

were conducted in 96-well microtiter plates in a total volume of 250 µL of buffer (50 mmol·L^{-1} Tris-HCl, pH 7.4). Non-specific binding was defined with 10 µmol·L^{-1} serotonin. The binding assays were initiated by the addition of 50 µL of the harvested transfected 5-HT$_{1A}$ cells (0.05 mg per sample) and were incubated at 25°C for 30 min. The reaction was terminated by vacuum filtration through presoaked (0.5% polyethyleneimine) Whatman GF/B filter paper (Brandel, Gaithersburg, MD, USA) using a Brandel 96-cell harvester. Filters were washed with ice-cold buffer (50 mmol·L^{-1} Tris-HCl, pH 7.4) and transferred to scintillation vials to which 5 mL of Opti-Fluor (Packard Instrument Company, Meriden, CT, USA) was added. Radioactivity was measured by liquid scintillation counting using a Beckman LS 6000TA liquid scintillation counter (Beckman Instruments, Fullerton, CA, USA). Protein concentrations were determined by the method of Bradford using bovine serum albumin as the standard. Binding data were analysed by ReceptorFit (Lundon Software, Cleveland Heights, OH, USA) a computer-assisted non-linear regression analysis program.

Functional activity: cAMP accumulation assay: Intracellular cAMP levels were measured using 96-well plates containing a stable transfection of intact human 5-HT$_{1A}$ receptors expressed in CHO cells. Upon initiation of the assay, the media from cell maintenance (i.e. Dulbecco's modified Eagle's solution containing 25 mmol·L^{-1} HEPES) was aspirated and cells were pre-incubated at 37°C for 15 min in Krebs buffer (NaCl 118 mmol·L^{-1}; KCl 5 mmol·L^{-1}; KH_2PO_4 1.2 mmol·L^{-1}; $NaHCO_3$ 25 mmol·L^{-1}; glucose 11.1 mmol·L^{-1}; $MgSO_4$ 1.2 mmol·L^{-1}; $CaCl_2$ 1.2 mmol·L^{-1}). Following this primary incubation, the buffer was aspirated and an additional incubation was performed at 37°C for 5 min in Krebs buffer containing 500 µmol·L^{-1} 3-isobutyl-1-methylxanthine. Subsequently, cells were incubated with 10 µmol·L^{-1} forskolin along with the compound to be tested for an additional 10 min at 37°C. For functional antagonism studies, antagonists were pre-incubated for 20 min prior to the addition of 10 nmol·L^{-1} 8-hydroxy-2-(di-n-propylamino)tertraline (8-OH-DPAT) and forskolin. All assays were terminated with the addition of 0.5 mol·L^{-1} perchloric acid. Intracellular cAMP levels were determined by radioimmunoassay through the cAMP scintillation proximity assay (SPA) screening kit. Data were analysed graphically with GraphPad Prism (GraphPad Software, San Diego, CA, USA). Non-linear regression analysis was used to determine IC_{50} values which were converted to K_B values using the method described by Cheng and Prusoff (1973). Note that agonists inhibit the forskolin-stimulated increase in cAMP accumulation, while antagonists such as WAY-100635 prevent 8-OH-DPAT from inhibiting the forskolin-induced increase in cAMP levels.

GTPγS assay: The [^{35}S]-GTPγS-binding assay was similar to that used by Lazareno and Birdsall (1993). Briefly, 5-HT$_{1A}$-cloned receptor membrane fragments (as used for 5-HT$_{1A}$ receptor-binding and cyclase assays) were stored at –70°C until needed. When needed, membranes were rapidly thawed, centrifuged at 40 000× *g* for 10 min and re-suspended at 4°C for 10 min in assay buffer (25 mmol·L^{-1} HEPES, 3 mmol·L^{-1} $MgCl_2$, 100 mmol·L^{-1} NaCl, 1 mmol·L^{-1} EDTA, 10 µmol·L^{-1} GDP, 500 mmol·L^{-1} dithiothreitol, pH 8.0). These membranes were then incubated for 30 min at 37°C with [^{35}S]GTPγS (1 nmol·L^{-1}) in the presence of vehicle, test compound or excess 8-OH-DPAT to define maximum agonist response. All reactions were terminated by the addition of ice-cold Tris buffer followed by rapid filtration using a Tom Tech® filtration device to separate bound from free [^{35}S]-GTPγS. Agonists produce an increase in the amount of [^{35}S]-GTPγS bound whereas antagonists produce no increase in binding. Bound radioactivity was counted and analysed as described above. Non-linear regression analysis was used to determine IC_{50} values which were converted to K_B values using the method described by Cheng and Prusoff (1973).

Secondary affinities: Selectivity of WAY-211612 for the α_1 adrenoceptor was determined by incubating rat cortical membranes with [^{3}H]-prazosin. Affinity for dopamine D_2, D_3 and D_4 receptors was determined using [^{3}H]-spiperone in CHO cells transfected with human dopamine D_2, D_3 and D_4 receptors. Affinity for WAY-211612 at various 5-HT receptor subtypes was assessed using CHO cells and [^{3}H]-5-HT for 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1F}$ and 5-HT$_{2B}$, [^{3}H]-DOI for 5-HT$_{2A}$ and 5-HT$_{2C}$ and [^{3}H]-LSD for 5-HT$_6$ and 5-HT$_7$ receptors. Standard filtration methodology was employed. Affinity for the human noradrenaline or the human dopamine transporter (DAT) was determined using [^{3}H]-nisoxetine (with desipramine defining non-specific binding) and [^{3}H]-WIN 35428 (with GBR-12935 defining non-specific binding) respectively. Recombinant human transporters (noradrenaline transporter and DAT) were expressed in CHO cells and binding was measured using SPAs. Non-linear regression analysis was used in all experiments to determine IC_{50} values that were converted to K_i values using the method of Cheng and Prusoff (1973).

In vivo *pharmacology*

Transporter and receptor occupancy studies. SERT. Male Sprague-Dawley rats (275–325 g) were used in all *in vivo* occupancy studies. Rats were dosed orally (5 mL·kg^{-1}) and received either vehicle (2% Tween80/0.5% methylcellulose), WAY-211612 (3 or 30 mg·kg^{-1}) or fluoxetine (30 mg·kg^{-1}). Thirty minutes later, rats received [^{3}H]- C-3-amino-4-[2-[(dimethylamino)methyl]phenylthio)-benzonitrile (DASB; 7.5 µCi in 0.3 mL saline) (specific activity = 84.6 µCi·mmole^{-1}; synthesized by Wyeth) injected through a lateral tail vein 30 min prior to death. Frontal cortex, hippocampus and cerebellum were dissected and weighed. NCS Tissue Solubilizer (GE Healthcare) was added to each sample at a concentration of 1 mL of solvent per 100 mg tissue and each sample was allowed to solubilize overnight with gentle shaking at 50°C. After approximately 16 h of shaking, 30 µL of glacial acetic acid was added per mL of solubilized tissue, to minimize background interference. The samples were cooled and an equal volume was removed from each solubilized tissue sample and transferred to a scintillation vial containing 15 mL of OptiFluor (PerkinElmer) and the tritium content determined by liquid scintillation spectrometry using a Canberra-Packard Tri-Carb 2200 liquid scintillation counter (PerkinElmer). SERT binding potential was determined for prefrontal cortex and hippocampus in each rat using the

cerebellum as a reference tissue for non-specific binding and to correct for possible differences in the amount of radioligand reaching the brain. The binding potential was calculated as (counts in prefrontal cortex – counts in cerebellum)/counts in cerebellum (Wadenberg *et al.*, 200). The binding potential value for the vehicle-treated group was pooled and the occupancy for each brain region of interest in each rat was then determined using the following formula: % receptor occupancy = 100[(SERT control-SERT individual region of interest)/SERT binding potential control] (Wadenberg *et al.*, 2000). Fluoxetine (30 mg·kg^{-1}, po) was used to define 100% occupancy.

5-HT$_{1A}$ receptors. Rats were dosed as above, with the exception that [^{3}H]-WAY-100635 (7.5 μCi in 0.3 mL saline; specific activity = 76.0 Ci·mmole^{-1}) (Amersham) instead of [^{3}H]-DASB was administered via the lateral tail vein. Hippocampus, frontal cortex, cerebellum and midbrain tissue containing dorsal raphe were dissected and processed and analysed as above with the exception that WAY-101405 (3 mg·kg^{-1}, po) was used as a positive control and produced near 100% occupancy with counts similar to cerebellar levels of binding.

In vivo *microdialysis*

Surgical preparation for microdialysis experiments. Male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighed between 280 and 350 g, at the time of surgery. All animals in the behavioural experiments were group housed in an AAALAC-accredited facility and maintained on a 12 h light/dark cycle. All experimentation was conducted during the light period (lights on at 0600 h). Halothane anesthesia (2–3%; Zeneca, Cheshire, UK) was administered before animals were secured in a stereotaxic frame with ear and incisor bars (David Kopf, Tujunga, CA, USA). A microdialysis guide cannula (CMA/12; CMA Microdialysis, Stockholm, Sweden) was directed above the dorsal lateral frontal cortex using the following coordinates: +3.2 mm anterior to bregma, –3.5 mm lateral from the midline and –1.3 mm ventral to dura with a flat skull (Paxinos and Watson, 1986). Guide cannulae were fixed to the skull with two stainless-steel screws (Small Parts, Roanoke, VA, USA) and dental acrylic (Plastics One, Roanoke, VA, USA). Following surgery, animals were individually housed in Plexiglas cages (45 cm sq.) for approximately 24 h and had access to food and water *ad libitum*.

Microdialysis and acute treatment with WAY-211612. Microdialysis procedures were performed as previously described (Beyer *et al.*, 2002). Concentric-style microdialysis probes (CMA/12; 20 kD cut-off) were purchased from CMA/Microdialysis and consisted of a 2-mm active membrane (OD 0.5 mm) and 14-mm stainless steel shaft (OD 0.64 mm). Probes were perfused with artificial cerebrospinal fluid (aCSF; 125 mmol·L^{-1} NaCl, 3 mmol·L^{-1} KCl, 0.75 mmol·L^{-1} $MgSO_4$ and 1.2 mmol·L^{-1} $CaCl_2$, pH 7.4) for at least 18 h according to the manufacturer's specifications. On the morning of experiments, microdialysis probes were inserted, via the guide cannula, into the dorsal lateral frontal cortex and perfused with aCSF at a flow rate of 1 μL·min^{-1}. A 3-hr stabilization period was allowed following probe insertion. Dialysis samples were collected every 20 min.

Initially, six dialysate samples were taken prior to drug injection to demonstrate a steady baseline. At the end of the sixth baseline sample, animals were given, orally, WAY-211613 (3–30 mg·kg^{-1}) or vehicle (2% Tween80), and dialysis samples were collected for the following 3 h. In a separate study, following baseline sample collection, animals received an injection of WAY-100635 (0.3 mg·kg^{-1}, s.c.) 20 min before fluoxetine (30 mg·kg^{-1}, s.c.). At the end of these experiments, animals were killed and probe placement was verified histologically.

Chronic treatment with WAY-211612. Prior to microdialysis cannula surgery, animals received a single injection of either WAY-211612 (30 mg·kg^{-1}, s.c.) or vehicle. Respective treatments were given for 14 consecutive days in the home cage of the animal. On day 15, animals underwent microdialysis surgery to implant a guide cannula above the dorsal lateral frontal cortex (see surgery section). No drug injections were given on the day of surgery. Similar to the acute studies, animals were allowed 24 h of post-operative recovery. The day after surgery (day 16), microdialysis studies were performed as described above and all animals received a challenge injection of WAY-211612 or vehicle. Based on their chronic treatment, three treatment groups were established on the day of the microdialysis experiment: vehicle (days 1–14) + vehicle (day 16, control); vehicle (days 1–14) + WAY-211612 (day 16, acute); WAY-211612 (days 1–14) + WAY-211612 (day 16, chronic).

Neurochemical analysis. The outlet tubing of the microdialysis probe was connected directly to an ANTEC (the Netherlands) HPLC system. In total, 20 μL dialysate containing 5-HT, noradrenaline and dopamine was separated by HPLC (C18 ODS3 column, 150 × 3.0 mm, Metachem, Torrance, CA, USA) and detected using an ANTEC electrochemical detector set at a potential of 0.65 V versus a Ag/AgCl reference electrode. The mobile phase (0.15 mol·L^{-1} NaH_2PO_4, 0.25 mmol·L^{-1} EDTA, 1.75 mmol·L^{-1} 1-octane sulphonic acid, 2% isopropanol and 4% methanol, pH = 4.6) was delivered by a Jasco PU1580 HPLC pump (Jasco Ltd, Essex, UK) at a flow rate of 0.5 mL·min^{-1}. Neurochemical data were compared with an external standard curve and all data was acquired using the Atlas software package (Thermo Labsystems, Beverley, MA, USA) for the PC. Neurotransmitter levels (fmol concentrations) collected during the baseline samples were averaged and this value was denoted as 100%. Subsequent sample values were expressed as a per cent change from this pre-injection baseline value (% change from baseline). Neurochemical data, excluding pre-injection values, were analysed by a two-way analysis of variance (ANOVA) with repeated measures (time). *Post hoc* analyses were made using the Bonferroni/Dunns adjustment for multiple comparisons. All statistical calculations were performed using the Statview software application (Abacus Concepts Inc., Berkeley, CA, USA) for the PC.

Rat resident-intruder model

Subjects. The procedures used were those described by Mitchell and Redfern (1997). Wistar rats were housed from

weaning (i.e. 3 weeks old) under reverse-daylight conditions (12 h on/12 h off, lights on 1900 h BST) for 5 weeks before the start of each experiment. Food and water was available *ad libitum*. The resident and intruder rats were obtained from different sources to ensure that resident animals had never been in contact with animals in the corresponding intruder group. All resident and intruder rats were housed in closed social groups of four rats per group for at least 5 weeks immediately before and throughout (intruder groups only) each experiment.

Resident-intruder paradigm. Resident animals were separated 3 days prior to each test day and housed individually with food and water available *ad libitum*. Thirty minutes prior to the social encounter, the resident rats were treated with either vehicle or WAY-211612 (3.3, 10.0 and 30 mg·kg^{-1}, s.c.). An unfamiliar intruder conspecific was then introduced into the resident rats' home cage and the ensuing social behaviour recorded onto videotape for 10 min. Following each social encounter, the resident and intruder rats were returned to their group cages. All drug solutions were prepared immediately prior to use and coded to ensure that the operator was unaware of each treatment administered to the resident rats.

Ethological analysis of behaviour. Ethological analysis of the resident rat's behaviour was always performed without knowledge of the treatment administered to each resident rat. From these records, the frequency of each behaviour/posture exhibited during each social encounter was calculated, grouped according to the motivational category in which that behaviour occurs, and the total score for each category was expressed as a percentage of the total number of behaviours observed for that animal. These categories of behaviours are represented in Table 1. Experience has shown that data arising from the ethological scoring method are generally positively skewed. All behavioural data were thus subjected to square root transformation before statistical analysis. All statistical procedures were performed using SuperANOVA (Abacus Concepts, Macintosh). Data were grouped according to treatment and the mean and standard error of the mean for both the percentage values of each motivational category and the total number of behaviours/postures calculated. The head/body shake data were also examined in detail separately. Data were subjected to repeated measures ANOVA across treatment for each category of behaviour. Where significant main effects of treatment were identified then significant differences from vehicle control treatment were determined using the Bonferroni/Dunn (control) *post hoc t*-test. In addition, the dose estimated to inhibit a particular behaviour by 50% (inhibitory dose 50%, ID_{50}) was calculated by the least squares method together with the appropriate 95% confidence limits.

Table 1 Rat resident-intruder model: summary of individual behaviours and postures*

Motivational category	*Behavioural element*
Exploration	Locomotion, rear
Maintenance	Wash, lick, scratch, shake, eat, drink, dig
Investigation	Approach, follow, stretched-attention, to fro, walk round/circle/side, nose and investigate, sniff genitalia, tail rattle
Sexual	Mount, attempt mount, lick penis
Aggression	Aggressive groom, aggressive posture, attack, bite, offensive sideways, offensive upright, pull, threat/thrust
Flight submit	Defensive sideways, defensive upright, submit
Flight escape	Attend, crouch, elevated crouch, flag and evade, retreat, under food hopper

*Behavioural postures are grouped according to motivational category exhibited by rats during social encounters (see Mitchell, 2005).

SIP

Individually housed male Sprague-Dawley rats (300–400 g) were maintained at approximately 85% of free feeding body weight. Rats were placed into an operant chamber (Med Associates, Vermont) and given free access to water. One food pellet was delivered into the food bin every minute for the duration of the procedure. This schedule of food delivery causes a repetitive drinking behaviour that results in the consumption of a large volume of water; generally five- to 10-fold greater than baseline, during the 120 min test session. On test days, WAY-211612 (30 and 56 mg·kg^{-1}, i.p.) or the combination of WAY-100635 (0.1 mg·kg^{-1}, i.p.) and fluoxetine (5.6 mg·kg^{-1}, i.p.) were given acutely to investigate effects on the adjunctive drinking behaviour. The per cent decrease in adjunctive drinking behaviour were analysed using a one-way ANOVA with a least significant difference (LSD) test as appropriate.

Four-plate assay

Male Swiss Webster mice (18–24 g) were housed in groups of 15 with free access to food and water. The 'four-plate' apparatus consists of a Plexiglas cage (18 × 25 × 16 cm) with a floor consisting of four identical rectangular metal plates (8 × 11 cm) separated from one another by a gap of 4 mm. The plates are connected to a device that generates electric shocks (0.8 mA, 0.5 s). Mice were given WAY-211612 (10–56 mg·kg^{-1}, po) 30 min before being placed individually into the four-plate apparatus. Initially, mice were allowed to habituate for 18 s. Following the 18 s habituation period, an electric shock (0.8 mA, 0.5 s) was delivered to the mouse when a crossing from one plate to another occurred. A 3 s time-out followed each shock and the number of punished crossings was recorded during a 1 min testing period. The total number of punished crossings were recorded by computer and analysed using a one-way ANOVA with a Dunnett's *post hoc* test as appropriate.

Materials

For the present series of experiments, Wyeth's Chemical and Screening Sciences group (Wyeth Research, Princeton, NJ, USA) synthesized WAY-211612 (Hatzenbuhler *et al.*, 2006) and its purity verified by standard analytical methods. The vehicle for WAY-211612 was 2% Tween 80/0.5% methylcellulose. All chemicals used for microdialysis and HPLC experiments were analytical grade and purchased from Sigma-Aldrich Chemicals (Milwaukee, WI, USA). All drug/molecular

Table 2 WAY-211612 is a dual SSRI/5-HT$_{1A}$ receptor antagonist

Compound	*5-HT Transporter*		*5-HT$_{1A}$ Receptors*		
	Binding	*Function*	*Binding*	*cAMP*	*GTPγS*
	(K_i, nmol·L^{-1})	*(K_B, nmol·L^{-1})*	*(K_i, nmol·L^{-1})*	*(K_B, nmol·L^{-1})*	*(K_B, nmol·L^{-1})*
WAY-211612	1.5 ± 0.1	17.7 ± 3.3	1.2 ± 0.2	6.3 ± 0.23	19.8 ± 2.6
Fluoxetine	3.9 ± 0.3	37.3 ± 2.9			
WAY-100635			1.0 ± 0.2	1.64 ± 0.3	3.4 ± 0.3

Values shown are means ± SEM. 5-HT transporter affinity was determined by competition with ^{3}H-paroxetine binding in rat cortical membranes and function was determined by the ability of compounds to inhibit ^{3}H-5-HT uptake into JAR cells expressing the human 5-HT transporter. 5-HT$_{1A}$ affinity and function were obtained using recombinant human 5-HT$_{1A}$ receptors. Receptor affinity was determined using ^{3}H-8-OHDPAT binding and antagonism was assessed using both an adenylate cyclase assay and a GTPγS assay. See methods for full details.
SSRI, 5-HT selective reuptake inhibitor.

target nomenclature in this paper conform to the British Journal of Pharmacology's Guide to Receptors and Channels (Alexander *et al*., 2008).

Results

In vitro pharmacology

WAY-211612 is a dual SSRI/5-HT$_{1A}$ receptor antagonist that is selective over a variety of other receptor systems. WAY-211612 is a novel molecule that has high affinity for SERTs (K_i = 1.5 nmol·L^{-1}) and 5-HT$_{1A}$ receptors (K_i = 1.2 nmol·L^{-1}; Table 2). A functional *in vitro* study revealed that WAY-211612 blocked [^{3}H] -5-HT uptake by human SERTs (K_B = 17.7 nmol·L^{-1}) and inhibited 5-HT$_{1A}$ agonist-induced cAMP accumulation (K_B = 6.3 nmol·L^{-1}) and 5-HT$_{1A}$-stimulated GTPγS^{35} binding (K_B = 19.8 nmol·L^{-1}) to human 5-HT$_{1A}$ receptors (Figure 1) WAY-211612 did not lower cAMP or GTPγS binding below basal levels, suggesting no inverse agonist actions. Table 2 also shows that WAY-211612 has similar affinity and function for the SERT and 5-HT$_{1A}$ receptor compared with the standard reference compounds fluoxetine (K_i = 3.9 nmol·L^{-1}; K_B = 37.3 nmol·L^{-1}) and WAY-100635 (K_i = 1.0 nmol·L^{-1}; cAMP accumulation, K_B = 1.6 nmol·L^{-1}; GTPγS^{35}, K_B = 3.4 nmol·L^{-1}). Apart from these primary activities, WAY-211612 exhibited more than 100-fold weaker activity at a variety of other receptors including α_1-adrenoceptors, dopamine D_2, D_3, D_4, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1F}$,5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_{5a}$, 5-HT$_6$ and 5-HT$_7$ receptors, and on the transporters for noradrenaline and dopamine (Table 3). Moreover, in a broad-panel Novascreen®, WAY-211612 lacked significant affinity at 63 additional sites for various other G-protein coupled receptors, enzymes and transporters (results not shown).

Transporter/5-HT$_{1A}$ receptor occupancy studies

WAY-211612 demonstrated significant SERT occupancy at 3 and 30 mg·kg^{-1}, po in both the prefrontal cortex and the hippocampus (Table 4). At the same doses, WAY-211612 produced less occupancy of 5-HT$_{1A}$ receptors (Table 4). At the lower dose of 3 mg·kg^{-1}, po, WAY-211612 produced minimal or no displacement of [^{3}H]-WAY-100635 bound to 5-HT$_{1A}$ receptors. However, at the higher dose, (30 mg·kg^{-1}, po) WAY-211612 did show considerable occupancy of the 5-HT$_{1A}$ receptors in prefrontal cortex, hippocampus and dorsal raphe. Note that due to the size of the dorsal raphe, the dissection also contained additional midbrain structures and had lower

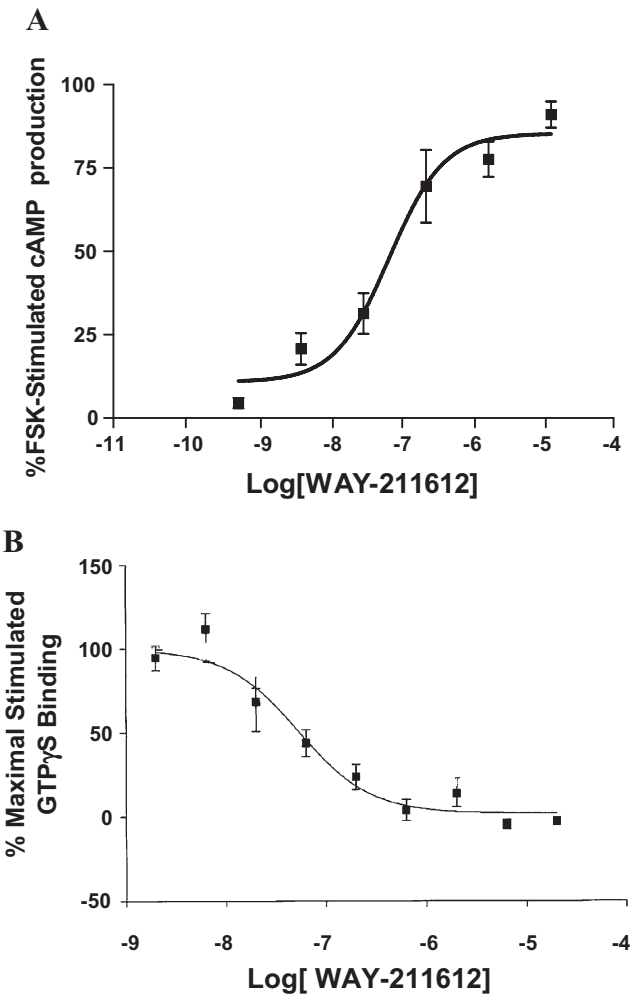

Figure 1 WAY-211612 was a full antagonist in the both the cAMP accumulation assay and in the GTPγS assay. Panel A illustrates the ability of WAY-211612 to reverse the 8-OH-DPAT-induced reduction in cAMP levels caused by forskolin stimulation. Panel B illustrates the ability of WAY-211612 to block 8-OH-DPAT-induced stimulation of GTPγS binding. All studies were performed using CHO cells expressing human 5-HT$_{1A}$ receptors. See methods for further details.

Table 3 WAY-211612 is selective over a variety of other receptors/transporters

Receptor	*K_i (nmol·L^{-1})*
Adrenoceptors	
α_1	>1000
α_{2A}	>1000[a]
Dopamine receptors	
D_2	>5000
D_3	>2344
D_4	452
5-HT receptors	
5-HT$_{1B}$	1057
5-HT$_{1D}$	>5000
5-HT$_{1F}$	>5000
5-HT$_{2A}$	847
5-HT$_{2B}$	565
5-HT$_{2C}$	769
5-ht$_{5a}$	>1000[a]
5-HT$_6$	2451
5-HT$_7$	747
Muscarininc receptors	
M_3, M_4, M_5	>1000[a]
Monoamine transporter	*K_i (nmol·L^{-1})*
hNET	>10 000 nmol·L^{-1}
hDAT	4800

Values of K_i were determined by incubating cells (in triplicate) with 9–11 concentrations of WAY-211612. In cases where less than 50% inhibition was observed at the highest concentration, K_i values are shown as >the highest concentration tested. All receptors were recombinant human receptors with the exception of the α_1 adrenoceptor, which was from rat cortical membranes.
[a]Results determined from selectivity assays with NovaScreen®, Hanover, MD.

Table 4 SERT and 5-HT$_{1A}$ receptor occupancy (%) by WAY-211612

Brain region	*Dose (po)*	*SERT*	*5-HT$_{1A}$*
Prefrontal cortex:	3 mg·kg^{-1}	56.6 ± 4.0	6.7 ± 4.0
	30 mg·kg^{-1}	73.6 ± 16.2	44.7 ± 2.4
Hippocampus:	3 mg·kg^{-1}	52.2 ± 4.4	8.3 ± 8.8
	30 mg·kg^{-1}	78.5 ± 13.2	48.6 ± 16.6
Dorsal raphe:	3 mg·kg^{-1}		14.8 ± 8.5
	30 mg·kg^{-1}		82.7 ± 13.6

Data in the Table show the displacement by WAY-211612 (as %) of paroxetine (SERT) or WAY-101405 (5-HT$_{1A}$ receptors). Values represent mean ± SEM. $n = 3$ for SERT and $n = 2$–3 for 5-HT$_{1A}$ receptors.
Note: Dorsal raphe dissection also contained some surrounding midbrain structures and was not purely dorsal raphe.
SERT, 5-HT transporter.

specific binding than the other regions examined. Receptor autoradiography would be best to quantitate receptor occupancy in this particular brain region.

Microdialysis studies

WAY-211612 acutely elevates cortical 5-HT levels in the rat frontal cortex. Acute administration of WAY-211612 (3–30 mg·kg^{-1}, po) rapidly and dose-dependently increased levels of 5-HT in the frontal cortex (Figure 2A). At 30 mg·kg^{-1}, maximal elevations in 5-HT were 75 ± 21% above baseline and occurred within 40 min of WAY-211612 administration. A one-way ANOVA with repeated measures (time) revealed a significant

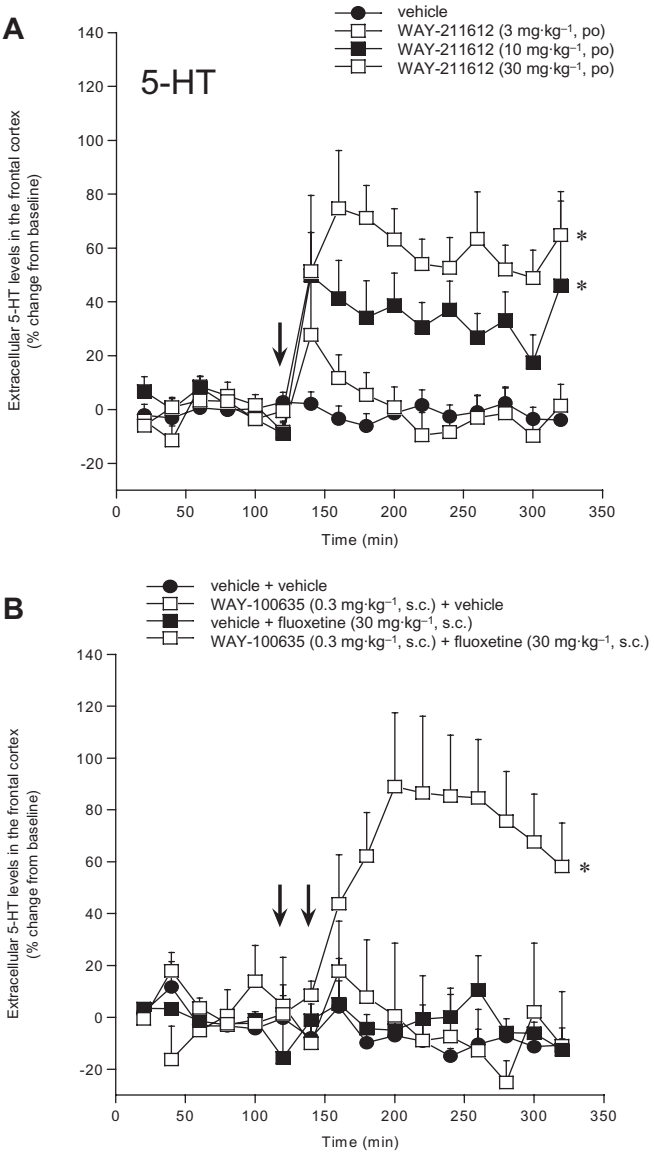

Figure 2 Acute administration of WAY-211612 increased the extracellular levels of 5-HT in the frontal cortex. Panel A illustrates that oral administration of WAY-211612 produced robust increases in cortical 5-HT at 10 mg·kg^{-1} ($n = 7$) and 30 mg·kg^{-1} ($n = 8$). The acute neurochemical effects of WAY-211612 were similar to that induced by a 20 min pretreatment with the selective 5-HT$_{1A}$ antagonist, WAY-100635 (0.3 mg·kg^{-1}, s.c.) and the SSRI, fluoxetine (30 mg·kg^{-1}, s.c.; closed square, $n = 8$, B). Consistent with previous reports, acute fluoxetine treatment alone (B, open square, $n = 7$) did not significantly affect extracellular 5-HT levels compared with vehicle controls. Asterisk (*) represents a significant ($P < 0.05$) treatment effect compared with vehicle.

increase in 5-HT at 10 and 30 mg·kg^{-1} [$F(3,33) = 13.02$, $P < 0.0001$] but not at the dose of 3 mg·kg^{-1} ($P = 0.7916$). As shown in Figure 2B, the acute effects of combining WAY-100635 (0.3 mg·kg^{-1}, s.c.) with fluoxetine (30 mg·kg^{-1}, s.c.; $F(2,21) = 10.44$, $P = 0.0007$] were to raise cortical 5-HT levels by 89 ± 28% above baseline, which was similar in magnitude and duration to the acute effects of WAY-211612. By comparison, and consistent with previous reports (see Discussion), fluoxetine treatment alone was insufficient to elevate extracellular 5-HT levels ($P = 0.4679$).

Chronic treatment with WAY-211612 preferentially increases cortical 5-HT levels. A challenge injection of WAY-211612 (30 mg·kg^{-1}, po) resulted in a significant increase in extracellular 5-HT in rats previously treated for 14 days with WAY-211612 [$F(2,19) = 21.49$, $P < 0.0001$; see Figure 3A]. This increase in 5-HT was similar to that elicited by acute administration of WAY-211612 in rats previously treated for 14 days with vehicle. A one-way ANOVA with repeated measures (time) revealed no significant difference between acute and chronic WAY-211612 treatment ($P = 0.1633$) indicating that tolerance does not develop to the neurochemical effects of this novel, dual-acting SSRI/5-HT$_{1A}$ receptor antagonist. No significant effects on basal (i.e. before WAY-211612 injection) levels of 5-HT were observed when comparing chronic vehicle (5-HT = 9.46 fmol·10 µL^{-1}) to chronic WAY-211612 (5-HT = 8.94 fmol·10 µL^{-1}) treatment (Student's *t*-test, $P = 0.759$; data not shown).

In contrast to the effects of 5-HT levels, neither acute nor chronic WAY-211612 (30 mg·kg^{-1}, po) treatment significantly altered extracellular levels of noradrenaline (see Figure 3B) or dopamine (see Figure 3C) in the rat frontal cortex [noradrenaline: $F(2,14) = 1.22$, $P = 0.3248$; dopamine: $F(2,16) = 0.82$, $P = 0.4579$]. Additionally, chronic treatment with WAY-211612 did not change rat body weight (data not shown).

Behavioural studies

WAY-211612 possesses antidepressant-like activity in the rat resident intruder model. The overall effects of acute administration of WAY-211612 on the behavioural profile of resident rats are represented in Table 5. Repeated measures ANOVA across treatment revealed significant main effects of acute WAY-211612 treatment on aggressive behaviour [$F(3,21) = 9.433$, $P = 0.0005$], flight escape behaviour [$F(3,21) = 4.114$, $P = 0.0192$] and head/body shake behaviour [$F(3,21) = 4.574$, $P = 0.0138$]. No other main effects on any other motivational category of behaviour were revealed [all $Fs(3,21) \leq 2.502$, $P \geq 0.05$ in all cases]. *Post hoc* analysis revealed that WAY-211612 dose-dependently reduced aggressive behaviour ($ID_{50} = 3.3$ mg·kg^{-1} s.c.) at a dose that had no effect on total behaviour score. *Post hoc* analysis also revealed that WAY-211612 significantly increased flight escape behaviour at 10 and 30 mg·kg^{-1} s.c. ($P < 0.05$ in both cases) but had no significant effect on cage exploration at any of the doses tested. Finally, resident rats treated with WAY-211612, at a dose of 30 mg·kg^{-1} only, exhibited significantly greater head/body shake behaviour (4.5 ± 1.8 shakes) compared with resident rats treated with drug vehicle (0.9 ± 0.4 shakes; $P < 0.05$).

WAY-211612 displays anti-obsessive compulsive disorder (OCD)-like activity in the rat SIP model. Systemic administration of WAY-211612 (30 and 56 mg·kg^{-1}, i.p.) decreased adjunctive drinking behaviour, an effect suggestive of anti-OCD-like activity (Figure 4A). A significant decrease in adjunctive drinking occurred when comparing vehicle treatment to WAY-211612 at the 56 mg·kg^{-1} dose [$F(2,14) = 7.23$, $P = 0.0087$]. *Post hoc* analysis using a LSD test revealed that at 56 mg·kg^{-1}, WAY-211612 significantly ($P = 0.0026$) decreased water intake, while at the 30 mg·kg^{-1} dose, WAY-211612 produced a strong trend towards decreasing water intake

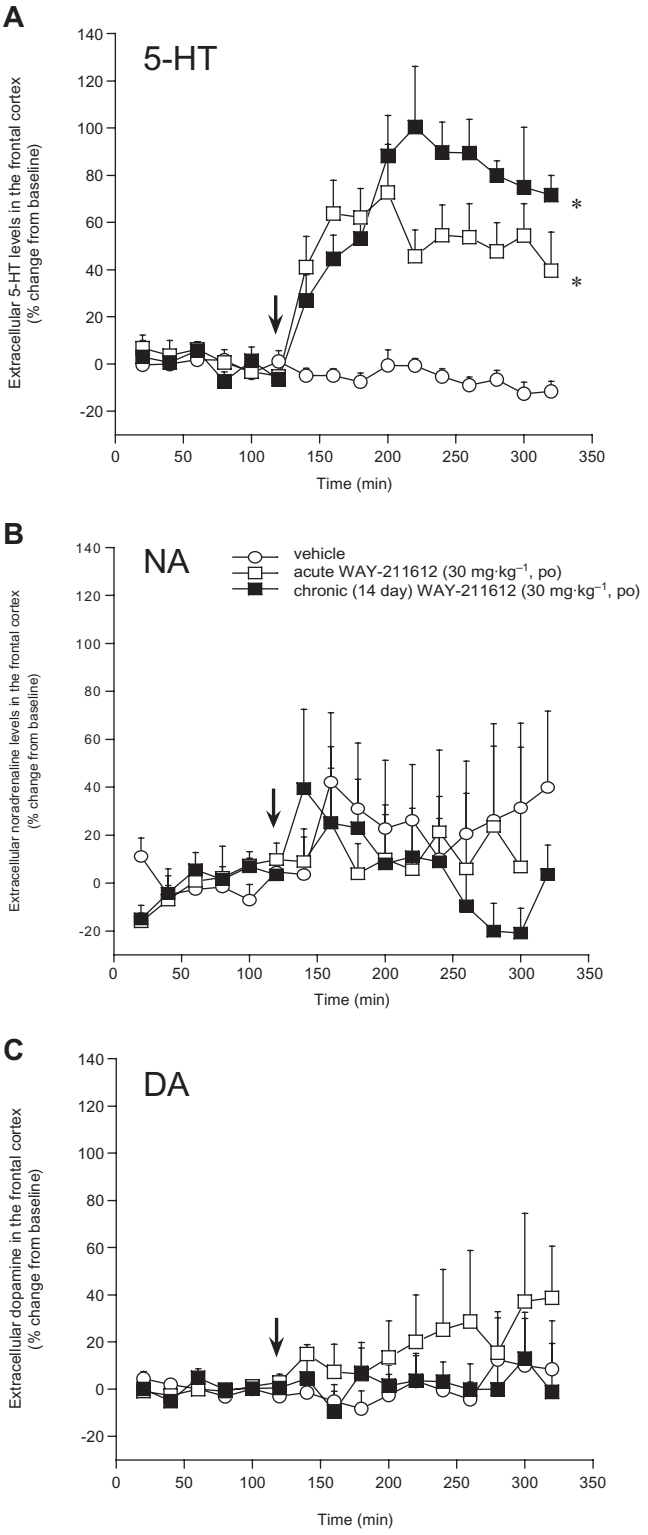

Figure 3 Chronic (14 day) administration of WAY-211612 (30 mg·kg^{-1}, po) increases 5-HT levels but not those of noradrenaline (NA) or dopamine (DA) in the rat frontal cortex. Panel A shows that acute ($n = 7$) and chronic ($n = 7$) treatment with 30 mg·kg^{-1} of WAY-211612 produces similar increases in extracellular 5-HT levels. In contrast, acute or chronic WAY-211612 treatment did not affect extracellular levels of noradrenaline (B) or dopamine (C) in the frontal cortex of the same animals. Asterisk (*) represents a significant ($P < 0.05$) treatment effect compared with vehicle.

Table 5 Effects of WAY-211612 (s.c.) on behaviours in the resident-intruder model

	Vehicle	*3.3 mg·kg^{-1}*	*10 mg·kg^{-1}*	*30 mg·kg^{-1}*
Exploration	24.5 ± 1.9	29.9 ± 2.9	31.7 ± 2.6	30.8 ± 1.7
Maintenance	1.4 ± 0.5	1.5 ± 0.2	1.7 ± 0.5	1.7 ± 0.3
Investigation	49.7 ± 2.3	45.5 ± 2.9	42.8 ± 3.1	43.5 ± 1.6
Sexual	0.9 ± 0.5	0.5 ± 0.2	0.6 ± 0.3	0.6 ± 0.2
Aggression	8.2 ± 1.1	4.2 ± 0.8*	3.3 ± 0.9*	3.0 ± 0.6*
Flight submit	1.4 ± 0.4	1.4 ± 0.3	2.1 ± 0.5	2.4 ± 0.6
Flight escape	13.9 ± 0.9	16.7 ± 0.9	17.8 ± 1.3*	17.9 ± 1.0*
Head/body shake	0.9 ± 0.4	1.6 ± 0.4	1.3 ± 0.4	4.5 ± 1.8*
Total behaviour	1664 ± 78	493 ± 126	1532 ± 92	1538 ± 43

Values indicate mean ± SEM percentage values of total behaviours for each motivational category except head/body shake and total behaviours are mean ± SEM of absolute values.
*$P < 0.05$; significantly different to vehicle treatment.

although this response did not achieve statistical significance ($P = 0.0561$). As observed with WAY-211612, combining WAY-100635 (0.1 mg·kg^{-1}, i.p.) and fluoxetine (30 mg·kg^{-1}, i.p.), at doses that did not elicit effects on water intake alone ($P = 0.3574$ and $P = 0.5976$ respectively), resulted in a significant [$F(3,25) = 6.028$, $P = 0.0037$] decrease in adjunctive drinking behaviour (Figure 4B).

WAY-211612 displays anxiolytic-like activity in the mouse four-plate test. WAY-211612 (3–30 mg·kg^{-1}, i.p.) displayed anxiolytic-like activity following acute administration (Figure 5A). An ANOVA revealed a significant increase in the number of punished crossings during the 1 min test session [$F(3,39) = 10.62$, $P < 0.0001$]. *Post hoc* analysis (LSD test) confirmed that 10 mg·kg^{-1} ($P = 0.0001$) and 30 mg·kg^{-1} ($P = 0.0002$), but not 3 mg·kg^{-1} ($P = 0.7133$), produced significant changes in the number of punished crossings compared with vehicle. Consistent with these effects, additional studies showed that, following oral administration, WAY-211612 (10–56 mg·kg^{-1}; Figure 4B) also significantly increased the punished crossings [$F(3,39) = 17.59$, $P < 0.0001$; LSD *post hoc* analysis), at all three doses evaluated ($P < 0.0001$).

Discussion

The delayed effect of conventional antidepressants is thought to result from the indirect inactivation (i.e. desensitization) of somatodendritic 5-HT$_{1A}$ autoreceptors (Artigas *et al.*, 1996; 2006). These observations provide strength to the hypothesis that a combination of an SSRI and a 5-HT$_{1A}$ receptor antagonist – a mechanism that would simultaneously block the SERT and mimic the desensitization of 5-HT$_{1A}$ receptors reported following chronic treatment – should elicit immediate increases in central 5-HT levels and yield an effective antidepressant action with an accelerated onset of activity. Herein, we report the pharmacological characterization of WAY-211612 (Hatzenbuhler *et al.*, 2006). This compound exhibited a dual mechanism of action as both an inhibitor of 5-HT reuptake ($K_i = 1.5$ nmol·L^{-1}; $K_B = 17.7$ nmol·L^{-1}) and a full antagonist ($I_{max} = 100\%$) at 5-HT$_{1A}$ receptors ($K_i = 1.2$ nmol·L^{-1}; $K_B = 6.3$ nmol·L^{-1} in the cAMP accumulation assay and 19.8 nmol·L^{-1} in GTPγS-binding assay). Moreover, when evaluated in a broad-panel Novascreen®, WAY-211612 was found to be at least 100-fold selective over a variety of other G-protein coupled receptors, enzymes and monoamine transporters.

While acute administration of SSRIs inhibits 5-hydroxytryptaminergic cell firing in the dorsal raphe nuclei through indirect activation of local somatodendritic 5-HT$_{1A}$ autoreceptors, chronic SSRI treatment is known to desensitize 5-HT$_{1A}$ autoreceptors and restore normal 5-hydroxytryptaminergic cell firing (Gartside *et al.*, 1999; Hervas *et al.*, 2001). These preclinical findings support the contention that SSRI-induced increases in forebrain 5-HT are negatively regulated by somatodendritic 5-HT$_{1A}$ autoreceptors (Blier and de Montigny, 1994; Romero and Artigas, 1997), which can be mimicked by competitively antagonizing 5-HT$_{1A}$ receptors. This is supported by neurochemical findings showing that co-administration of selective 5-HT$_{1A}$ receptor antagonists, such as WAY-100635, augment 5-HT levels evoked by SSRIs (Hjorth *et al.*, 1997; Sharp *et al.*, 1997; Dawson and Nguyen, 1998; Beyer *et al.*, 2002). Consistent with this hypothesis, a single injection of WAY-211612 dose-dependently elevated extracellular levels of 5-HT in the rat frontal cortex, an effect shown to be similar in magnitude to acute treatment with WAY-100635 and fluoxetine (comparing Figure 2A to 2B) and to the neurochemical effects elicited by chronic SSRI treatment (Kreiss and Lucki, 1995; Dawson and Nguyen, 1998). Thus, in an area of the rat frontal cortex (i.e. the dorsal lateral cortex) which has been shown to only be sensitive to the chronic – but not the acute – 5-hydroxytryptaminergic effects of SSRIs (Beyer and Cremers, 2008), WAY-211612 elicited a rapid and acute neurochemical response that may be consistent with a fast-acting antidepressant agent.

One question to consider when interpreting the present microdialysis results is whether WAY-211612 is functioning as a full or a partial, 5-HT$_{1A}$ receptor antagonist. However, there are several results that would argue against the latter interpretation. For instance, partial 5-HT$_{1A}$ receptor agonists such as buspirone (Dawson and Nguyen, 1998), tandospirone (Yoshino *et al.*, 2002) and, in some cases, pindolol (Hughes and Sharp, 1998), fail to produce the same potentiation in SSRI-induced 5-HT levels. The microdialysis results of the present study confirm that WAY-211612 is not acting as a partial agonist at 5-HT$_{1A}$ receptors as acute treatment with this molecule evoked robust increases in cortical levels of 5-HT

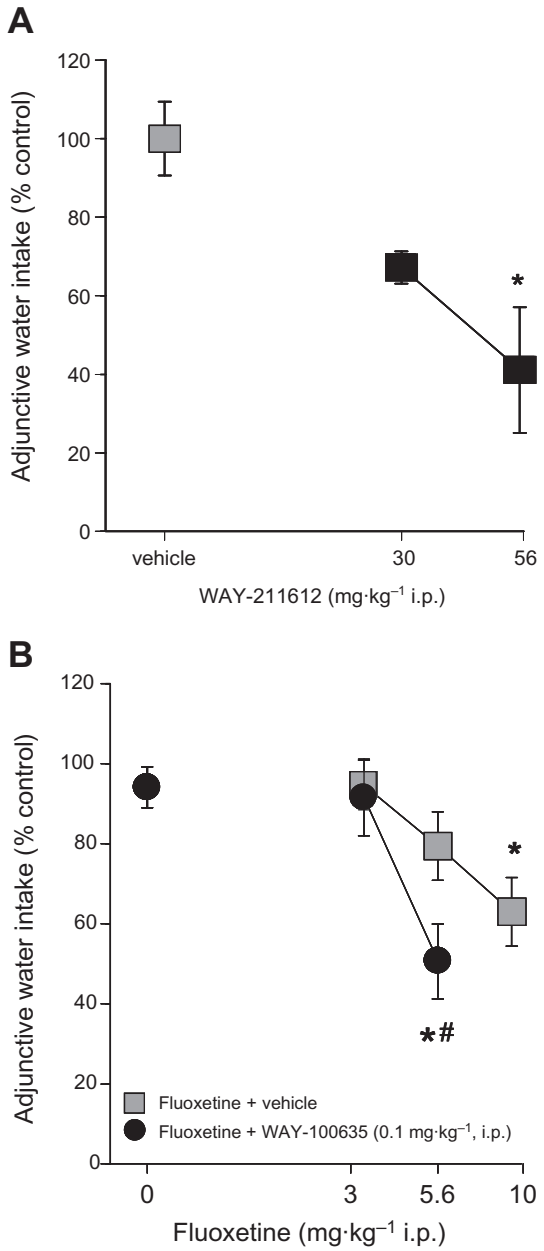

Figure 4 WAY-211612 displays anti-OCD-like activity in the rat SIP model. Panel A illustrates that systemic administration of WAY-211612 (30 and 56 mg·kg^{-1}, i.p.) decreased adjunctive drinking behaviour, an effect suggestive of anti-OCD-like activity. Similarly, combining WAY-100635 (0.1 mg·kg^{-1}, i.p.) and fluoxetine (30 mg·kg^{-1}, i.p.), at doses that did not elicit effects on water intake alone, resulted in a significant decrease in adjunctive drinking behaviour (B). *$P < 0.05$ compared with vehicle; #$P < 0.05$ compared with fluoxetine treatment. OCD, obsessive compulsive disorder.

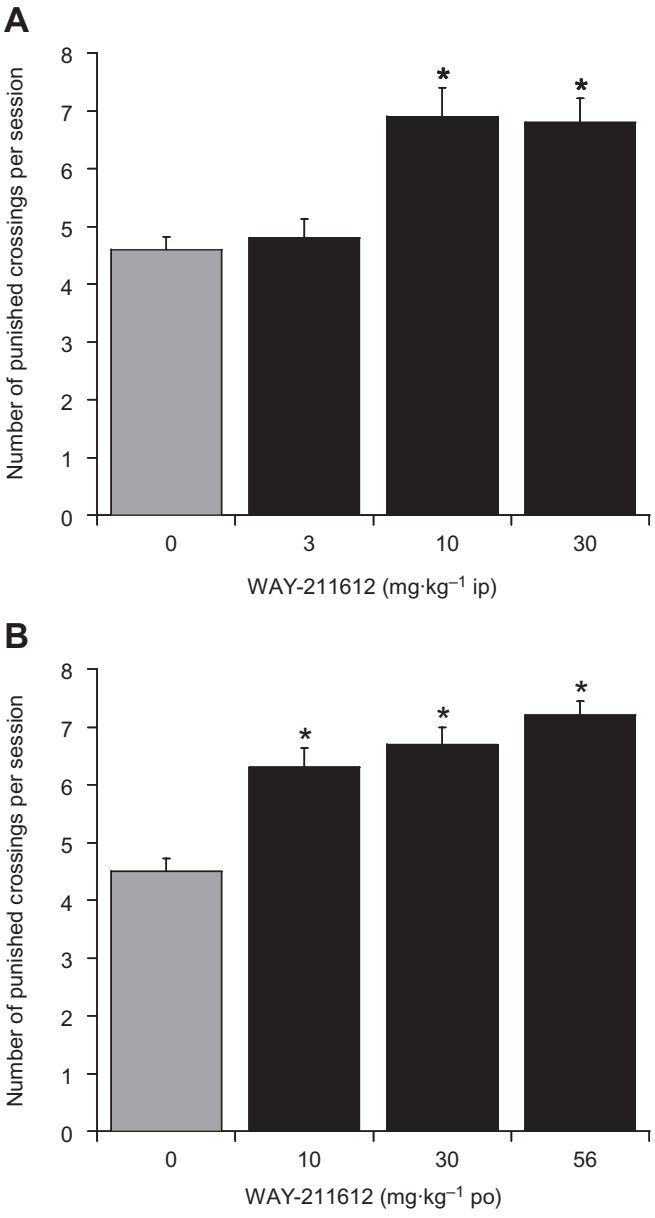

Figure 5 Anxiolytic-like activity of WAY-211612 in the mouse four-plate test. Intraperitoneal injection of WAY-211612 (3–30 mg·kg^{-1}) produced significant increases in punished crossing during the 1 min test session (A, $n = 10$). Panel B shows that WAY-211612 is orally active in the four-plate model (n-10). *$P < 0.05$ compared with vehicle.

following acute treatment. This hypothesis is further supported by the fact that WAY-211612 did not alter cortical levels of noradrenaline or dopamine in the current study. Thus, while selective and full 5-HT$_{1A}$ antagonists are not reported to affect noradrenaline or dopamine levels (Beyer *et al*., 2002), previous microdialysis studies demonstrated that partial 5-HT$_{1A}$ receptor agonists elicit marked increases in extracellular noradrenaline and dopamine levels in awake (Gobert *et al*., 1998) or anesthetized (Done and Sharp, 1994) rodents. The involvement of both primary targets (SERT and 5-HT$_{1A}$ receptors) is further supported by our current results showing that maximal effects in the microdialysis experiments was seen after a dose of 30 mg·kg^{-1}, po and that dose was associated with significant occupancy of both 5-HT$_{1A}$ receptors (44.7%, 48.6% and 82.7% in prefrontal cortex, hippocampus and dorsal raphe respectively) and SERTs (73.6% and 78.5% respectively in prefrontal cortex and hippocampus). Additionally, there was minimal occupancy of 5-HT$_{1A}$ receptors (6.7%, 8.3% and 14.8% in prefrontal cortex, hippocampus and dorsal raphe respectively) at a dose of WAY-211612

that was ineffective in the microdialysis experiments (3 mg·kg^{-1}). It should be noted that due to the small size of the dorsal raphe, the tissues used for quantitation of occupancy also likely contained other midbrain regions. Although the current results show clear occupancy of dorsal raphe 5-HT$_{1A}$ receptors at 30 mg·kg^{-1} of WAY-211612, receptor autoradiography would be required to determine if there were differential occupancy of these receptors versus 5-HT$_{1A}$ receptors in the prefrontal cortex or hippocampus.

As the neurochemical profile of WAY-211612 was suggestive of a novel antidepressant, we examined the potential antidepressant-like activity of WAY-211612 in the rat resident intruder model. In these studies, acute treatment with WAY-211612 elicited a dose-dependent reduction in the level of aggressive behaviour exhibited by resident rats. Importantly, this response occurred at doses of WAY-211612 that concomitantly increased flight behaviour. Furthermore, these changes in agonistic behaviour were observed at doses that had no effect on the total number of behaviours exhibited by the resident rats indicating that the reduced aggression was a specific change in behaviour that was not simply the result of non-specific drug effects, such as sedation. The preclinical results of antidepressant-like activity of WAY-211612 are similar to findings with pharmacologically disparate antidepressant drugs (including tricyclic antidepressants, monoamine oxidase inhibitors, selective inhibitors of 5-HT or noradrenaline uptake, 5-HT$_{1A}$ receptor agonists and partial agonists, 5-HT$_{2C}$ receptor agonists) in this model (Mitchell, 2005). The lowering of aggressive behaviour in resident rats is most likely to reflect the ability of WAY-211612, as well as other antidepressants, to increase central 5-hydroxytryptaminergic activity *in vivo*. Thus, even at the dose of 3 mg·kg^{-1}, WAY-211612 produced an approximate 25% increase in cortical 5-HT. Although this latter effect was not statistically significant, it may indicate that only a small concentration in 5-HT is required to mediate these behavioural responses. Moreover, WAY-211612 (at the highest dose of 30 mg·kg^{-1}) significantly increased rodent head/body shake behaviour; a phenomena most likely mediated by 5-HT-induced stimulation of central 5-HT$_{2A}$ receptors (Pranzatelli, 1990; Schreiber *et al*., 1995).

WAY-211612 was further evaluated in the rat SIP model, a paradigm proposed to be suitable in assessing the onset of antidepressant action (Woods *et al*., 1993; Hogg and Dalvi, 2004), as well as a model of OCD (Hogg and Dalvi, 2004). In this model, acute treatment with WAY-211612 dose-dependently decreased adjunctive drinking behaviour. Importantly, animals in these studies consumed all the food pellets delivered during the test (data not shown), indicating that the effects of WAY-211612 in this model were not likely the result of non-specific behavioural/appetitive effects. These results are consistent with reports that 5-hydroxytryptaminergic mechanisms such as combining WAY-100635 with fluoxetine reduce drinking effects in the SIP model (Figure 3B; Woods *et al*., 1993; Hogg and Dalvi, 2004). Interestingly, in those studies, fluoxetine treatment significantly reduced responses in SIP only after 5–6 days of treatment, compared with the combination of WAY-100635 and fluoxetine, which decreased SIP effects on the first day of treatment (Figure 3B; Hogg and Dalvi, 2004). Similarly, WAY-211612 suppressed adjunctive drinking behaviour following acute administration. These present data, taken together with previous work in this model (Woods *et al*., 1993; Hogg and Dalvi, 2004), suggest that WAY-211612 exhibited anti-OCD-like activity and may also be a molecule with an accelerated onset of antidepressant activity. This contention, in addition to being supported by the acute neurochemical effects in the dorsal lateral cortex (see Beyer and Cremers, 2008), is also supported by previous reports that WAY-100635 accelerated the time-dependent, antidepressant-like effects of SSRIs in the rodent resident intruder model (Mitchell and Redfern, 1997).

The mouse four-plate assay is routinely used as preclinical model of anxiety (Hascoet *et al*., 2000; Ring *et al*., 2006; Malberg *et al*., 2007). In this model, acute treatment with WAY-211612 significantly increased the number of punished crosses, a response indicative of anxiolytic-like activity. To our knowledge, there are no published reports available combining SSRIs with 5-HT$_{1A}$ receptor antagonists in the mouse four-plate assay. However, these results are consistent with previous reports that SSRIs, including citalopram, fluovoxamine and paroxetine, and the mixed SSRI/noradrenaline reuptake inhibitors, venlafaxine and milnacipran, acutely increased the number of punished crossings in the mouse four-plate assay (Hascoet *et al*., 2000). Furthermore, previous reports suggest that selective 5-HT$_{1A}$ receptor antagonists display anxiolytic-like activity on their own in this model (Wesolowska *et al*., 2003). Overall, the ability of WAY-211612 to increase punished crossings in the four-plate model is consistent with this compound's ability to preferentially increase 5-HT transmission and/or block 5-HT$_{1A}$ receptors. Moreover, the present data suggest that WAY-211612 possesses anxiolytic-like activity *in vivo*, which is also consistent with the clinical use of SSRIs in the treatment of anxiety disorders (reviewed in Golden, 2004).

The combination of SSRI/5-HT$_{1A}$ antagonist treatment has been evaluated in many clinical studies. Pindolol, which represents the only 5-HT$_{1A}$ antagonist approved for use in humans, has been shown to accelerate the antidepressant effects of SSRIs in a meta-analysis of 15 clinical and open-label studies (Artigas *et al*., 1994; Blier and Bergeron, 1995; Ballesteros and Callado, 2004), although some investigations have not confirmed these results (Berman *et al*., 1997). While there are caveats to consider when interpreting these findings – for example, pindolol is a β-adrenoceptor antagonist which has 5-HT$_{1A}$ partial agonist properties in rats *in vivo* (Gartside *et al*., 1999) – this strategy has helped to further substantiate the 5-hydroxytryptaminergic hypothesis of depression. It is also worth noting that previous work has shown that 5-HT$_{1A}$ agonists also may possess antidepressant effects (Cryan *et al*., 1997). Additionally, the impact of 5-HT$_{1A}$ receptor agonism/antagonism will depend greatly on the tonic activation of 5-HT$_{1A}$ receptors in depressed patients. Therefore, it remains to be fully evaluated whether partial agonists or the more purely selective 5-HT$_{1A}$ antagonists such as the recently described lecozotan (Schechter *et al*., 2005) and robalzotan (Arborelius *et al*., 1999), when combined with antidepressants, will have clinical success in treating depressed patients. If this hypothesis is confirmed in the clinic, compounds like WAY-211612, and related dual-acting compounds like vilazodone (Hughes *et al*., 2005), are likely to represent a new

generation of effective antidepressants that may also possess an accelerated onset of activity.

In conclusion, the present series of experiments characterize a novel molecule displaying potent and selective dual SSRI/5-HT$_{1A}$ antagonist activity. The acute treatment with WAY-211612 produced *in vivo* occupancy of both SERTs and 5-HT$_{1A}$ receptors and resulted in robust elevations in cortical 5-HT levels similar to those seen after a combination of WAY-100635 and fluoxetine. Given that WAY-211612 increases 5-HT in the dorsal lateral cortex – an area of the rat frontal cortex that is sensitive to the chronic, but not the acute, 5-hydroxytryptaminergic effects of SSRIs (Beyer and Cremers, 2008) – these neurochemical findings suggest that acute treatment with WAY-211612 may possess rapid antidepressant effects similar to that of chronic SSRI treatment. The neurochemical response of WAY-211612 was specific for 5-HT, as no changes in noradrenaline or dopamine were observed. Moreover, tolerance did not develop to the 5-hydroxytryptaminergic effects of WAY-211612 following chronic (14 day) treatment. If these exciting preclinical findings ultimately translate into clinical efficacy, WAY-211612, and related compounds, could represent the next generation of novel and effective antidepressants.

Conflict of interest

The following authors were full-time employees of Wyeth Pharmaceuticals when these experiments were performed or when this manuscript was written and revised: Chad E. Beyer, Qian Lin, Brian Platt, Jessica Malberg, Geoffrey Hornby, Kelly M. Sullivan, Deborah L. Smith, Tim Lock, Nicole T. Hatzenbuhler, Deborah A. Evrard, Boyd L. Harrison, Ronald Magolda, Menelas N. Pangalos, Lee E. Schechter, Sharon Rosenzweig-Lipson, Terrance H. Andree.

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