

## RESEARCH PAPER

# Involvement of the $\sigma_1$ ( $\sigma_1$ ) receptor in the anti-amnesic, but not antidepressant-like, effects of the aminotetrahydrofuran derivative ANAVEX1-41

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**Background and purpose:** Tetrahydro-N, N-dimethyl-5, 5-diphenyl-3-furanmethanamine hydrochloride (ANAVEX1-41) is a potent muscarinic and  $\sigma_1$  ( $\sigma_1$ ) receptor ligand. The  $\sigma_1$  receptor modulates glutamatergic and cholinergic responses in the forebrain and selective agonists are potent anti-amnesic and antidepressant drugs. We have here analysed the  $\sigma_1$  component in the behavioural effects of ANAVEX1-41.

**Experimental approach:** Binding of ANAVEX1-41 to muscarinic and  $\sigma_1$  receptors were measured using cell membranes. Behavioural effects of ANAVEX1-41 were tested in mice using memory (spontaneous alternation, passive avoidance, water-maze) and antidepressant-like activity (forced swimming) procedures.

**Key results:** *In vitro*, ANAVEX1-41 was a potent muscarinic ( $M_1 > M_3$ ,  $M_4 > M_2$  with  $K_i$  ranging from 18 to 114 nM) and selective  $\sigma_1$  ligand ( $\sigma_1$ ,  $K_i = 44$  nM;  $\sigma_2$ ,  $K_i = 4$   $\mu$ M). In mice, ANAVEX1-41 failed to affect learning when injected alone (0.03–1 mg kg<sup>-1</sup>), but attenuated scopolamine-induced amnesia with a bell-shaped dose response (maximum at 0.1 mg kg<sup>-1</sup>). The  $\sigma_1$  antagonist BD1047 blocked the anti-amnesic effect of ANAVEX1-41 on both short- and long-term memories. Pretreatment with a  $\sigma_1$  receptor-directed antisense oligodeoxynucleotide prevented effects of ANAVEX1-41 only in the passive avoidance procedure, measuring long-term memory. ANAVEX1-41 reduced behavioural despair at 30 and 60 mg kg<sup>-1</sup>, without involving the  $\sigma_1$  receptor, as it was not blocked by BD1047 or the antisense oligodeoxynucleotide.

**Conclusions and implications:** ANAVEX1-41 is a potent anti-amnesic drug, acting through muscarinic and  $\sigma_1$  receptors. The latter component may be involved in the enhancing effects of the drug on long-term memory processes.

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**Keywords:** ANAVEX1-41; muscarinic receptors; sigma-1 receptors; learning and memory; depression; spontaneous alternation; passive avoidance; forced swimming; scopolamine

**Abbreviations:** ANAVEX1-41, tetrahydro-N,N-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride; BD1047, N-[2-(3, 4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino) ethylamine hydrochloride; DMEM, Dulbecco's modified Eagle's medium; DTG, 1,3-di-o-tolylguanidine; ER, endoplasmic reticulum; i.c.v., intracerebroventricular; O, opposite quadrant; ODN, oligodeoxynucleotide; S1R asODN,  $\sigma_1$  receptor-targeted antisense oligodeoxynucleotide; S1R mODN,  $\sigma_1$  receptor-targeted mismatch oligodeoxynucleotide

## Introduction

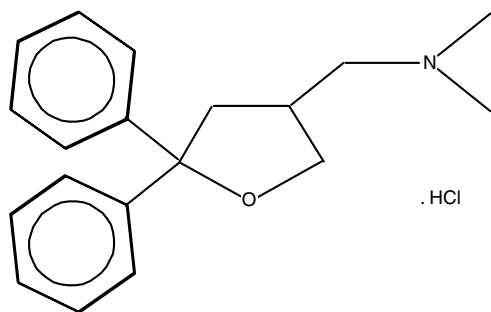
Tetrahydro-N,N-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride (ANAVEX1-41, formerly AE14, Figure 1) is a new tetrahydrofuran compound showing prominent anti-amnesic, anticonvulsant and anti-depressant potential (Vamvakides, 2002). Preliminary experiments revealed that

ANAVEX1-41, at 0.3 and 3 mg kg<sup>-1</sup> per os (p.o.), improved the amnesic capacities of mice submitted to a step-down type passive avoidance procedure. ANAVEX1-41, at 19 or 45 mg kg<sup>-1</sup> p.o., antagonized, respectively, pentylenetetrazol- or electroshock-induced tonic seizures without any sedative action. Moreover, increased activity, not always significant, was observed in mice, in the same dose range. From neurochemical studies in rat brain (nucleus accumbens, striatum, hippocampus, cortex and hypothalamus), this hypermobility was not sustained by central dopaminergic hyperactivity (unpublished data). These observations, if

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**Figure 1** Structure of tetrahydro-*N,N*-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride (ANAVEX1-41).

confirmed by other *in vitro* and *in vivo* studies, suggest there could be interesting effects of ANAVEX1-41 in addiction models. The compound also shortened the duration of immobility in the single, 6-min duration forced swimming test at 10–50 mg kg<sup>-1</sup> intraperitoneally (i.p.) or 30–100 mg kg<sup>-1</sup> p.o. (Vamvakides, 2002).

Several pharmacological targets have been identified for ANAVEX1-41. In particular, pharmacological screening using competition binding confirmed that ANAVEX1-41 is a potent muscarinic acetylcholine receptor ligand and a  $\sigma_1$  receptor ligand. Functional tests in the rabbit vas deferens, guinea-pig atria and ileum revealed that the drug is an M<sub>1</sub> muscarinic acetylcholine receptor agonist and an M<sub>2</sub>/M<sub>3</sub> receptor antagonist, with a median efficacy of 1  $\mu$ M (for M<sub>1</sub> and M<sub>2</sub>) and 0.3  $\mu$ M (for M<sub>3</sub>). The compound also inhibited binding of [<sup>3</sup>H]batrachotoxin to the sodium channel site 2, with micromolar affinities (Vamvakides, 2002). Micromolar affinities were also found for  $\alpha_1$ -adrenoceptors, 5-HT<sub>2</sub> and even dopamine D<sub>3</sub> receptors (at 10  $\mu$ M) (Vamvakides, 2002). ANAVEX1-41 may therefore present a very attractive pharmacological profile with unique characteristics. The drug may simultaneously act on membrane-bound muscarinic receptors and intracellular  $\sigma_1$  receptors, which are mainly located on the endoplasmic reticulum (ER) (Hayashi *et al.*, 2000). Consequently, synergistic mechanisms could be expected from such effects of ANAVEX1-41 on muscarinic and  $\sigma_1$  receptors.

The aim of the present study was to establish firmly whether ANAVEX1-41 readily interacts with muscarinic and  $\sigma_1$  receptors *in vivo*. The binding profile of the drug on muscarinic and  $\sigma$  receptors was first determined. The promnesic effects of ANAVEX1-41 alone or the anti-amnesic effects of the drug in combination with scopolamine were then examined in mice submitted to short-term memory tests (spontaneous alternation) or long-term, spatial or contextual, memory tests (place learning in the water-maze and passive avoidance, respectively). The antidepressant-like effect of the compound was examined using the forced swimming test. Finally, the involvement of the  $\sigma_1$  receptor in the drug effect was examined using pretreatment with the  $\sigma_1$  receptor antagonist BD1047 (*N*-[2-(3, 4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino) ethylamine hydrochloride) or repeated injections of an antisense oligodeoxynucleotide (asODN) probe, as described previously (Maurice *et al.*, 2001b).

## Methods

### Animals

Male Swiss mice (from the breeding centre of the Faculty of Pharmacy, Montpellier, France), aged 7 weeks and weighing 32 ± 2 g were used in this study. Animals were housed in plastic cages in groups. They had free access to food and water, except during behavioural experiments, and they were kept in a regulated environment (23 ± 1°C, 40–60% humidity) under a 12-h light/dark cycle (light on at 0800 hours). Experiments were carried out between 0900 and 1700 hours, in an experimental room within the animal facility. Mice were habituated 30 min before each experiment. All animal procedures were conducted with strict adherence to the European Union Directive of 24 November 1986 (86–609).

### Design and administration of oligodeoxynucleotides

Based on the mouse cDNA sequence for the  $\sigma_1$  receptor, 16-mer phosphorothioate-modified oligodeoxynucleotide (ODN) sequences were designed, as described previously (Maurice *et al.*, 2001b). They were targeted to the area from +15 to +1 around the initiation codon, 5'-CGCGGCCACGCGCATT-3' (= antisense ODN,  $\sigma_1$  receptor-targeted antisense oligodeoxynucleotide (S1R asODN)). As a control, a mismatched analogue, including randomly designed defects, 5'-CACGTCCCTCTCCATT-3', was designed (= mismatch ODN,  $\sigma_1$  receptor-targeted mismatch oligodeoxynucleotide (S1R mODN)). ODN were synthesized and purified by high-pressure liquid chromatography by Eurobio Laboratoires (Les Ulis, France). They were dissolved in sterile distilled water and stored at -20°C until used.

Mice were anaesthetized using intramuscular administration of ketamine (80 mg kg<sup>-1</sup>) and xylazine (10 mg kg<sup>-1</sup>). A polyethylene cannula (0.75 mm inner diameter and 6 mm length) was implanted and fixed using acrylic cement. The tip of the cannula was placed in the right ventricle, with stereotaxic coordinates from the Bregma being, in mm, A -0.5, L -1, V 2.5. Injections began 24 h after surgery. The needle of a Hamilton microsyringe was inserted through the cannula, and ODN (1  $\mu$ l) were slowly injected over 1 min followed by an additional 1 min wait before removing the needle. Animals received two intracerebroventricular (i.c.v.) injections per day, at 12 h time interval, during 3 days. They were used for behavioural observations, 10 h after the last injection, that is 4 days after implanting the cannula.

### Binding assays to muscarinic receptors

**Cell culture.** Chinese hamster ovary (CHO-K1) cells, stably transfected with the human M<sub>1</sub>–M<sub>4</sub> mAChR, were kindly provided by Dr M Brann (University of Vermont Medical School, Burlington, VT, USA). Cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 20 mM HEPES, 10% fetal bovine serum and 50  $\mu$ g ml<sup>-1</sup> geneticin, and were grown for 4 days at 37°C in a humidified incubator containing 5% CO<sub>2</sub>:95% O<sub>2</sub>. Cells were then harvested by trypsinization followed by centrifugation (300 g, 3 min) and resuspension of the pellet in HEPES buffer (110 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl<sub>2</sub>, 1 mM

MgSO<sub>4</sub>, 25 mM glucose, 50 mM HEPES, 58 mM sucrose, pH 7.4), repeated twice.

**Cell membrane preparation.** For membrane-based radioligand binding assays, CHO cells were grown, harvested and centrifuged as described above, with the final pellet resuspended in 5 ml of ice-cold homogenization buffer (50 mM HEPES, 2.5 mM MgCl<sub>2</sub>, 2 mM EGTA), and then homogenized using a Polytron homogenizer for three 10 s intervals at maximum setting with 30 s cooling periods employed between each burst. The homogenate was centrifuged (1000 g, 10 min, 25°C), the pellet discarded and the supernatant was re-centrifuged at 30 000 g for 30 min at 4°C. The resulting pellet was resuspended in 5 ml of HEPES buffer and protein content was determined using the method of Bradford (1976). The homogenate was then divided into 1 ml aliquots and either used immediately or stored frozen at -80°C until required for radioligand binding assays.

**[<sup>3</sup>H]NMS inhibition binding assays.** Inhibition binding studies were performed by incubating CHO M<sub>1</sub>-M<sub>4</sub> cell membranes (10–20 mg protein per tube) with [<sup>3</sup>H]NMS (0.2 nM) and a range of concentrations of ANAVEX1-41 at 37°C for 1 h in HEPES buffer. The final assay volume was 0.5 ml. Nonspecific binding was defined as that remaining in the presence of 10 mM atropine. The reaction was terminated by rapid filtration through Whatman GF/B filters, which were then washed with 5 × 1 ml ice-cold 0.9% (w/v) NaCl solution and allowed to dry before bound radioactivity was measured using liquid scintillation counting.

#### *Binding assays to $\sigma$ receptors*

The  $\sigma_1$  binding assay was performed, according to Ganapathy *et al.* (1999), by incubating Jurkat cell membranes (10–20 mg protein per tube) with [<sup>3</sup>H](+)-pentazocine (8 nM) and a range of concentrations of ANAVEX1-41 at 22°C for 2 h in 5 mM Tris/HCl buffer, pH = 7.4. The  $\sigma_2$  binding assay was performed, according to Bowen *et al.* (1993), by incubating rat cerebral cortex membranes (10–20 mg protein per tube) with [<sup>3</sup>H](+)-DTG (1,3-di-*o*-tolylguanidine; 5 nM) in the presence of (+)-pentazocine (300 nM), to saturate  $\sigma_1$  site binding, and a range of concentrations of ANAVEX1-41 at 22°C for 2 h in 5 mM Tris/HCl buffer, pH = 7.4. The final assay volume was 0.5 ml. Nonspecific binding was defined, in both assays, as that remaining in the presence of 10  $\mu$ M haloperidol. The reaction was terminated by rapid filtration through Whatman GF/B filters, which were then washed with 5 × 1 ml ice-cold 0.9% (w/v) NaCl solution and allowed to dry before bound radioactivity was measured using liquid scintillation counting. The protein concentration in the homogenates was determined using the method of Bradford (1976).

#### *Forced swimming test*

Each mouse was placed in a glass cylinder (diameter 12 cm and height 24 cm) filled with water at a height of 12 cm. Water temperature was maintained at 22–23°C. The animal was forced to swim for 15 min on the first day. On the second day, each mouse was placed again into the water and forced to swim for 6 min. The session was videotaped and the duration

of immobility during the last 5 min was measured. The mouse was considered as immobile when it stopped struggling and moved only to remain floating in the water. Drugs were administered 30 min before the session on the second day.

#### *Spontaneous alternation performances*

Each mouse, naive to the apparatus, was placed at the end of one arm in a Y-maze (three arms, 50 cm long, 60° separate) and allowed to move freely through the maze during a single 8-min session. The series of arm entries, including possible returns into the same arm, was recorded visually. An alternation was defined as entries into all three arms on consecutive trials. The number of the total possible alternations was therefore the total number of arm entries minus two, and the percentage of alternation was calculated as (actual alternations/total possible alternations) × 100. Animals performing less than eight arm entries in 8 min were discarded (that is, less than 5% of animals). The compounds were administered 30 min before the session or 10 min before scopolamine, given 20 min before the session.

#### *Step-down passive avoidance test*

The apparatus consisted of a transparent acrylic cage (30 × 30 × 40 cm high) with a grid-floor, inserted in a soundproof outer box (35 × 35 × 90 cm high). The cage was lit by a 15 W lamp during the experimental period. A wooden platform (4 × 4 × 4 cm) was fixed at the centre of the grid-floor. Intermittent electric shocks (1 Hz, 500 ms, 40 V DC) were delivered to the grid-floor using an isolated pulse stimulator (Model 2100, AM Systems, Everett, WA, USA). The test comprised two training sessions, at 90-min time interval, and a retention session, carried out 24 h after the first training. During training sessions, each mouse was placed on the platform. When it stepped down and placed its four paws on the grid-floor, shocks were delivered for 15 s. Step-down latency and the numbers of vocalizations and flinching reactions were measured. Shock sensitivity was evaluated by adding these two numbers. None of the treatments used in the present study significantly affected the shock sensitivity. Animals that stepped down before 3 s has elapsed or that did not step down within 30 s were discarded (that is, less than 5% of the mice). Animals, which did not step down within 60 s during the second session, were considered as remembering the task and removed without receiving further electric shocks. This procedure, used routinely, permits the intra-group variability to be minimized without affecting the relevance of the behavioural measure. The retention test was performed in a similar manner as training, except that the shocks were not applied to the grid-floor. Each mouse was again placed on the platform, and the latency recorded, with an upper cut-off time of 300 s. Two parametric measures of retention were analysed: the latency and the number of animals reaching the avoidance criterion, defined as correct if the latency measured during the retention session was greater than threefold the latency showed by the animal during the second training session and, at least, greater than 60 s. ANAVEX1-41 and/or BD1047 were administered 30 min and scopolamine 20 min

before the first training, and injections were not repeated before the second training or the retention test.

#### Place learning in the water-maze

The maze was a circular pool (160 cm diameter, 40 cm height) that the videotracking systems could arbitrarily divide into four quadrants. The water temperature ( $24 \pm 2^\circ\text{C}$ ), light intensity, external cues in the room, and water opacity, obtained by a suspension of calcium carbonate, were controlled and rigorously reproduced. A transparent Plexiglas platform, 10 cm in diameter, could be immersed 2 cm under the water surface at the centre of each quadrant during training sessions. This quadrant was termed the training (T) quadrant and the others opposite (O), adjacent right (AR) and adjacent left (AL) quadrants, during the subsequent retention session. Swimming was recorded using a CCD camera connected to a computer, trajectories being analysed in terms of latencies and distances, and thus swim speed, using the Videotrack software (View-point, Champagne-au-Mont-d'Or, France).

The behavioural procedure was carried out as follows. Mice were trained to learn a fixed location of the invisible platform during 5 days. They were then submitted to a probe test with the platform being removed from the pool. Training consisted of three swimming sessions per day with a 20 min inter-trial time interval. Start positions, set at each limit between quadrants, were randomly selected for each animal. Each animal was allowed a 90 s swim to find the platform and was left for a further 30 s on the platform. Animals failing to find the platform were placed on it manually. On day 5, 2 h after the last swim, animals were submitted to a probe test. The platform was removed and each mouse was allowed a free 60 s swim. The percentage of time spent in each quadrant was determined. Then, animals were submitted to a working memory procedure with platform location changing every day, during 3 days, as described previously (Yamada *et al.*, 1999; Meunier and Maurice, 2004). The testing procedure was similar to that described previously, except that mice received four trials per day with 2 min inter-trial time interval, and the platform location changed every day but not among trials. Data represent the mean performance over days for each trial. During each swimming session, the animal is allowed to swim to the platform in its new location. Mice show a progressive involvement of the working memory between each trial, therefore decreasing the latency spent to reach the platform, and the quality of spatial working memory was examined by focussing on the difference in swimming duration between trials 1 and 4.

#### Statistical analyses

Radio-ligand inhibition binding isotherms for the interaction between [ $^3\text{H}$ ]radio-ligand and ANAVEX1-41 were fitted according to the following one-site mass-action equation:

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + \frac{[I]}{K_i \left(1 + \frac{[A]}{K_A}\right)}}$$

where Y denotes the percent-specific binding, Top and Bottom denote the maximal and minimal asymptotes,

respectively, [A] denotes the concentration of [ $^3\text{H}$ ]radio-ligand, [I] denotes the unlabeled ANAVEX1-41 concentration, and  $K_A$  and  $K_i$  denote the equilibrium dissociation constants of A and I, respectively. For all analyses, the negative logarithm of the  $K_A$  value for [ $^3\text{H}$ ]NMS was fixed as 9.6, as determined separately in saturation binding assays; for [ $^3\text{H}$ ](+)-pentazocine as 7.9, according to Ganapathy *et al.* (1999); and for [ $^3\text{H}$ ]DTG as 7.6, according to Bowen *et al.* (1993).

Behavioural data were expressed as means  $\pm$  s.e.m., except step-down latencies which were expressed as medians and interquartile ranges. They were analysed using one-way analysis of variance (ANOVA; F-values) or two-way ANOVA with the treatment or dose and ODN as independent factors, followed by the Dunnett's *post hoc* multiple comparison test. Passive avoidance latencies were analysed with Kruskal-Wallis non-parametric ANOVA (KW values), since upper cut-off times were set, followed by the Dunn's multiple comparisons test. Performances over time in the water-maze were analysed using the non-parametric Friedman ANOVA (Fr values), followed by the Dunn's multiple comparisons test. The level of statistical significance was  $P < 0.05$ .

#### Drugs

Tetrahydro-*N,N*-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride (ANAVEX1-41, formerly AE14, Figure 1) was synthesized in the laboratory (Anavex Life Sciences, Pallini, Greece), according to the methodology and characterization described previously (Kolocouris *et al.*, 1985; Vamvakides *et al.*, 1997). *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino)ethylamine hydrochloride (BD1047) was kindly provided by Dr Wayne D Bowen (Brown University, Providence, RI, USA). Haloperidol was from Janssen (Boulogne-Billancourt, France). [ $^3\text{H}$ ]N-Methylscopolamine methyl chloride ([ $^3\text{H}$ ]NMS, 2.59–3.2 TBq mmol $^{-1}$ ), [ring-1,3-3H](+)-pentazocine ([ $^3\text{H}$ ](+)-pentazocine, 0.925–2.22 TBq mmol $^{-1}$ ) and [p-ring-3H]([ $^3\text{H}$ ]DTG, 1.11–2.22 TBq mmol $^{-1}$ ) were from Perkin Elmer Life Science (Boston, MA, USA). DMEM and geneticin were purchased from Life Technologies Gibco BRL (Grand Island, NY, USA). Fetal bovine serum was purchased from ThermoTrace (Melbourne, Victoria, Australia). All other materials, including scopolamine hydrobromide, were purchased from Sigma-Aldrich (St Louis, MO, USA or St-Quentin Fallavier, France). Drugs used for *in vivo* experiments were dissolved in physiological saline solution and administered i.p. or subcutaneously (s.c.) in a volume of 100  $\mu\text{l}$  per 20 g body weight.

## Results

#### Binding affinities of ANAVEX1-41 for muscarinic and $\sigma$ sites

Measures of the binding affinities of ANAVEX1-41 for muscarinic binding sites showed that the compound is a very potent  $M_1$  ligand ( $K_i = 18.5$  nM; Table 1). The compound also binds to  $M_3$  and  $M_4$  subtypes with a high affinity ( $K_i = 50$  and 77 nM, respectively; Table 1). ANAVEX1-41 binds to  $M_2$  sites with a more moderate affinity ( $K_i = 114$  nM;

**Table 1** Binding affinities of ANAVEX1-41 on muscarinic and  $\sigma$  sites

Receptor sites	Radioligand	$K_i$ (nM)
M <sub>1</sub> muscarinic	[ <sup>3</sup> H]NMS	18.5 ± 1.8
M <sub>2</sub> muscarinic	[ <sup>3</sup> H]NMS	114.4 ± 9.6
M <sub>3</sub> muscarinic	[ <sup>3</sup> H]NMS	50.1 ± 1.1
M <sub>4</sub> muscarinic	[ <sup>3</sup> H]NMS	77.0 ± 12.9
$\sigma_1$	[ <sup>3</sup> H](+)-pentazocine	44.4 ± 3.0
$\sigma_2$	[ <sup>3</sup> H]DTG	3924 ± 186

Data are mean ± s.e.m. of 2–4 determinations.

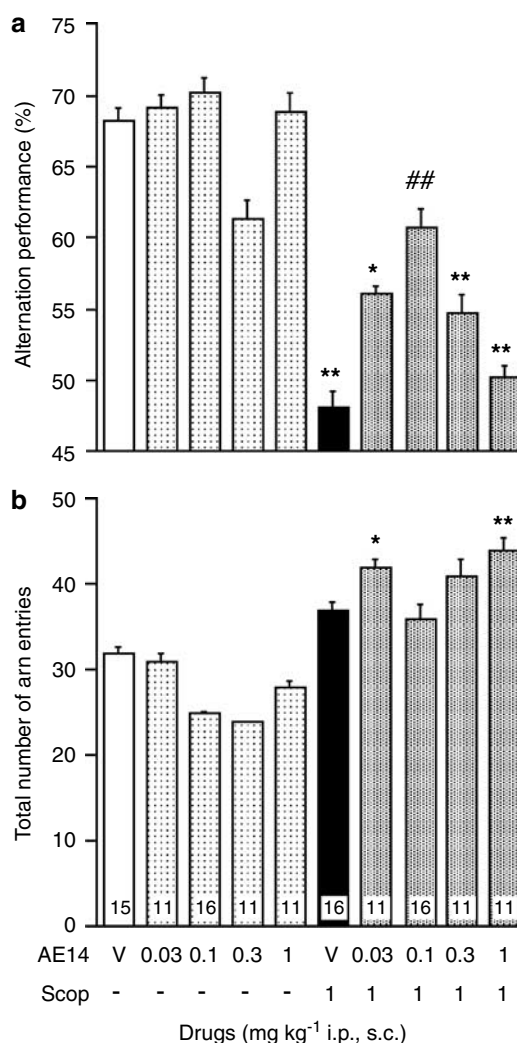
Table 1). The compound is also a potent  $\sigma_1$  site ligand, with a  $K_i$  value of 44 nM in the concentration range of its M<sub>1</sub>/M<sub>3</sub>/M<sub>4</sub> affinities. Interestingly, ANAVEX1-41 is very selective for  $\sigma_1$  vs  $\sigma_2$  site ( $K_i$  = 3.9  $\mu$ M, selectivity factor: 88; Table 1).

#### Anti-amnesic effects of ANAVEX1-41 against scopolamine-induced learning impairments

The memory effects of ANAVEX1-41 were tested in a series of behavioural procedures assessing different memory processes. The compound was tested alone and as pretreatment before scopolamine, a muscarinic receptor antagonist that significantly impedes learning. First, mice were tested for spontaneous alternation performance in the Y-maze, an index of spatial working memory (Maurice *et al.*, 1994). Second, animals were examined in a step-down type passive avoidance procedure, assessing contextual long-term memory (Maurice *et al.*, 1994). Third, mice were tested for place learning in the water-maze using a procedure consisting first of the acquisition of a fixed platform location during 5 days, involving both working and reference memory; and second, a procedure comprised 3 days of acquisition of a daily changing platform position, a procedure exclusively involving the spatial working memory component (Yamada *et al.*, 1999; Meunier and Maurice, 2004).

During the Y-maze session, two parameters were measured: the alternation percentage (Figure 2a) and number of arm entries (Figure 2b), an index of exploratory activity in the maze. The i.p. administration of ANAVEX1-41, in the 0.03–1 mg kg<sup>-1</sup> dose range, alone failed to affect the alternation percentage. Scopolamine, at 1 mg kg<sup>-1</sup> s.c., significantly decreased the alternation performance. Pre-treatment with ANAVEX1-41 allowed a bell-shaped attenuation of the scopolamine-induced deficits with a significant effect noted for the dose of 0.1 mg kg<sup>-1</sup> (Figure 2a). In parallel, the scopolamine treatment increased nonsignificantly the number of arm entries, a tendency strengthened by the ANAVEX1-41 pretreatment, since significant differences appeared with two doses of the drug (Figure 2b).

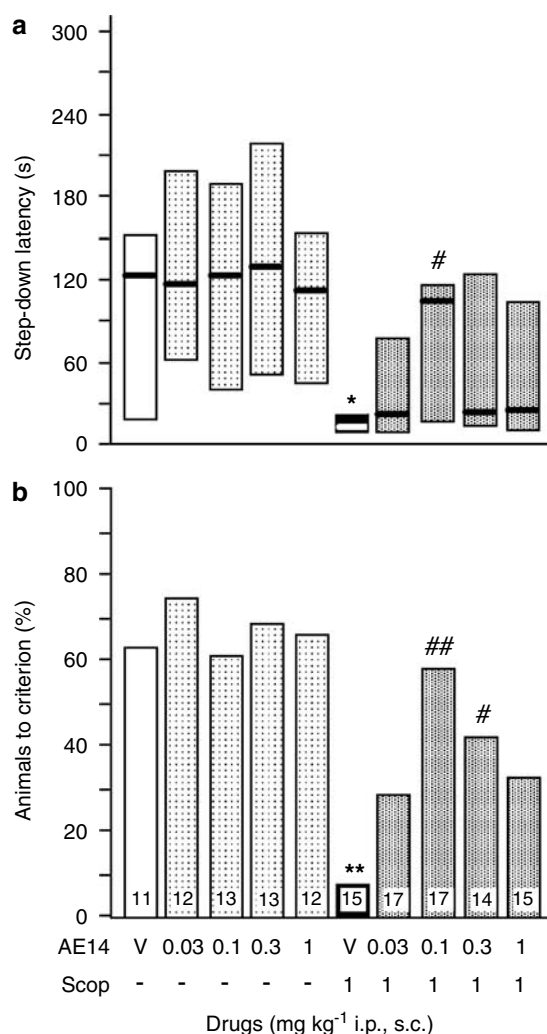
During the passive avoidance retention session, two parameters were analysed: the step-down latency (Figure 3a) and percentage of animals reaching the avoidance criterion (Figure 3b). Administration of ANAVEX1-41 alone failed to affect both parameters, suggesting that the compound failed to enhance the mnemonic ability in this procedure. The scopolamine injection significantly affected both the latency and percentage of animals reaching the avoidance criterion (animals-to-criterion; Figures 3a and b). Pre-treatment with ANAVEX1-41 allowed a bell-shaped



**Figure 2** Dose-related effects of ANAVEX1-41 (AE14) on alternation behaviour in scopolamine-treated mice: (a) alternation performances and (b) total number of arm entries. ANAVEX1-41 (0.03–1 mg kg<sup>-1</sup> i.p.) or vehicle solution (V) was administered 30 min before the test, or 10 min before scopolamine (Scop, 1 mg kg<sup>-1</sup> s.c.), which was administered 20 min before the test. The number of mice per group is indicated in (b);  $F(9,119) = 8.62$ ,  $P < 0.0001$  in (a);  $F(9,119) = 7.04$ ,  $P < 0.0001$  in (b). \* $P < 0.05$ , \*\* $P < 0.01$  vs the V-treated group; ## $P < 0.01$  vs the Scop-treated group; Dunnett's test. ANAVEX1-41, tetrahydro-*N,N*-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride; i.p., intraperitoneally; s.c., subcutaneously.

attenuation of the scopolamine-induced deficits, with a significant effect noted for the dose of 0.1 mg kg<sup>-1</sup> (Figure 3a). At the doses of 0.1 and 0.3 mg kg<sup>-1</sup>, the scopolamine-induced decrease in animals-to-criterion was significantly attenuated (Figure 3b).

ANAVEX1-41 was tested at a single dose in the water-maze test. The 0.1 mg kg<sup>-1</sup> i.p. dose was selected, as being the most effective dose in the two previous tests. Four experimental groups were tested in parallel: mice receiving saline solution i.p. and s.c.; mice receiving ANAVEX1-41, 0.1 mg kg<sup>-1</sup> i.p. and saline s.c.; mice receiving saline i.p. and scopolamine 1 mg kg<sup>-1</sup> s.c.; and animals receiving both ANAVEX1-41 i.p. and scopolamine s.c. It must be noted that the treatments did not affect the swimming speed. Calculations of the swimming speed during



**Figure 3** Dose-related effects of ANAVEX1-41 (AE14) on the scopolamine-induced amnesia in the step-down passive avoidance test: (a) step-down latency and (b) percentage of animals to criterion. ANAVEX1-41 (0.03–1 mg kg<sup>-1</sup> i.p.) or vehicle solution (V) was administered 30 min before the first training, or 10 min before scopolamine (Scop, 1 mg kg<sup>-1</sup> s.c.), which was administered 20 min before the first training. A second training was repeated 90 min after, and retention was examined after 24 h. Step-down latencies are expressed as median and interquartile range in (a). The number of mice per group is indicated in (b); KW = 31.0,  $P < 0.001$ . \* $P < 0.05$ , \*\* $P < 0.01$  vs the V-treated group; # $P < 0.05$ , ## $P < 0.01$  vs the Scop-treated group; Dunn's test in (a) and  $\chi^2$ -test in (b). ANAVEX1-41, tetrahydro-*N,N*-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride; i.p., intraperitoneally; s.c., subcutaneously.

day 5 sessions gave:  $29.5 \pm 1.0 \text{ cm s}^{-1}$  for the vehicle-treated group,  $29.1 \pm 1.2 \text{ cm s}^{-1}$  for the scopolamine-treated group,  $28.2 \pm 0.8 \text{ cm s}^{-1}$  for the ANAVEX1-41-treated group and  $27.6 \pm 1.3 \text{ cm s}^{-1}$  for the (ANAVEX1-41 + scopolamine)-treated group ( $F(3,111) = 0.60$ ,  $P > 0.05$ ). Results could therefore be analysed either as time to reach the platform or distance to reach the fixed platform position. As shown in Figure 4a, vehicle-treated mice show a progressive decrease in time to reach the platform over training sessions (trials 2 and 4,  $P < 0.05$  vs trial 1; trials 3 and 5,  $P < 0.001$ ). The ANAVEX1-41 treatment failed to alter the performances over time (trials

3–5,  $P < 0.01$  vs trial 1; Figure 4a). The scopolamine treatment, however, resulted in a worsening of performances (trials 3–5,  $P < 0.05$  vs trial 1; Figure 4b). In particular, significant augmentations of escape latencies were measured during trials 3–5 as compared with the vehicle-treated control group (Figure 4b). The ANAVEX1-41 pre-administration resulted in an amelioration of the performances over time (trial 2,  $P < 0.05$  vs trial 1; trials 3–5,  $P < 0.01$ ; Figure 4b). Indeed, escape latencies measured during trials 3 and 4 for the (ANAVEX1-41 + scopolamine)-treated group were significantly lower as compared with the time to reach the platform showed by the scopolamine-treated group (Figure 4b).

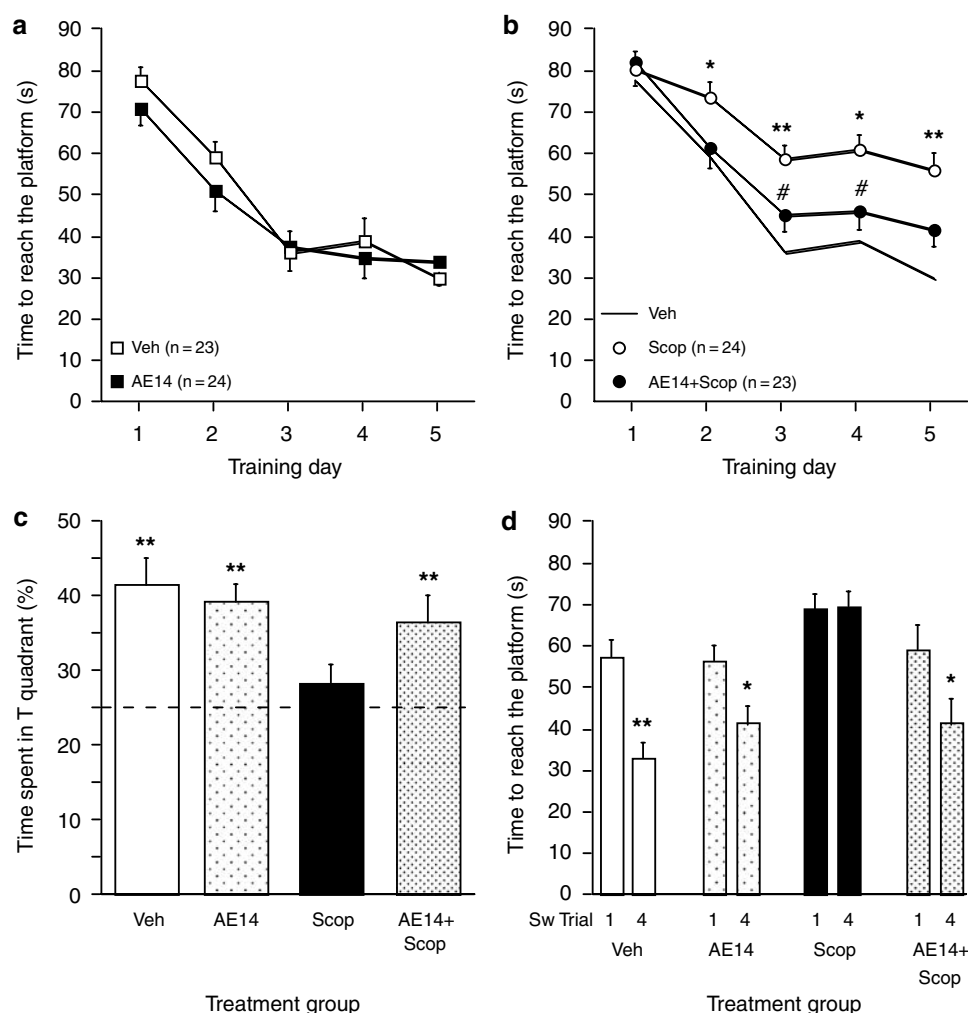
During the probe test performed 2 h after the last swimming trial, a preferential presence in the training (T) quadrant vs the chance level was measured for all groups, except for the scopolamine-treated group (Figure 4c). In particular, the (ANAVEX1-41 + scopolamine)-treated group showed a significantly increased duration of swimming in the training quadrant, as compared with scopolamine-treated animals, indicating that the ANAVEX1-41 pretreatment blocked the scopolamine-induced impairment.

Animals were then submitted to 3 days of acquisition of a daily changing location of the platform. Results were analysed in terms of decrease in escape latency between the 1st and 4th swimming trials (Figure 4d). This decrease remained non-significantly different for the control group, but reached significance for the ANAVEX1-41 and (ANAVEX1-41 + scopolamine) groups (Figure 4d). On the contrary, the scopolamine-treated group showed no change in latency among swimming trials. Results from the probe test and working memory procedure, therefore, confirmed that scopolamine impeded spatial learning in the water-maze, an impairment affecting both the working and reference memory processes. The scopolamine-induced deficit could be prevented by a pretreatment with 0.1 mg kg<sup>-1</sup> ANAVEX1-41.

#### *An interaction with the $\sigma_1$ receptor is involved in the anti-amnesic effects of ANAVEX1-41*

To address this point, we performed two series of experiments. First, antagonism studies were carried out using the  $\sigma_1$  receptor antagonist BD1047, administered simultaneously with ANAVEX1-41. Second, mice received a repeated i.c.v. treatment with an antisense ODN targeting the  $\sigma_1$  receptor (Maurice *et al.*, 2001b) and the anti-amnesic effect of ANAVEX1-41 was then assessed.

BD1047, administered alone over a range of doses (0.03–1 mg kg<sup>-1</sup> i.p.), failed to affect the alternation performance or exploratory behaviour in the Y-maze (Figures 5a and b). Moreover, when injected before scopolamine, the compound failed to affect the amnesic and hyperactive effects of the muscarinic antagonist (Figures 5a and b). However, when co-administered with the active dose of ANAVEX1-41 before scopolamine, BD1047 blocked the anti-amnesic effect of ANAVEX1-41 (Figure 5c), without any significant effect in terms of exploratory response (Figure 5d). The antagonism was significant at the dose of 0.1 mg kg<sup>-1</sup> i.p. (Figure 5c). Similar results were obtained in the passive avoidance procedure. BD1047 administered alone failed to affect the

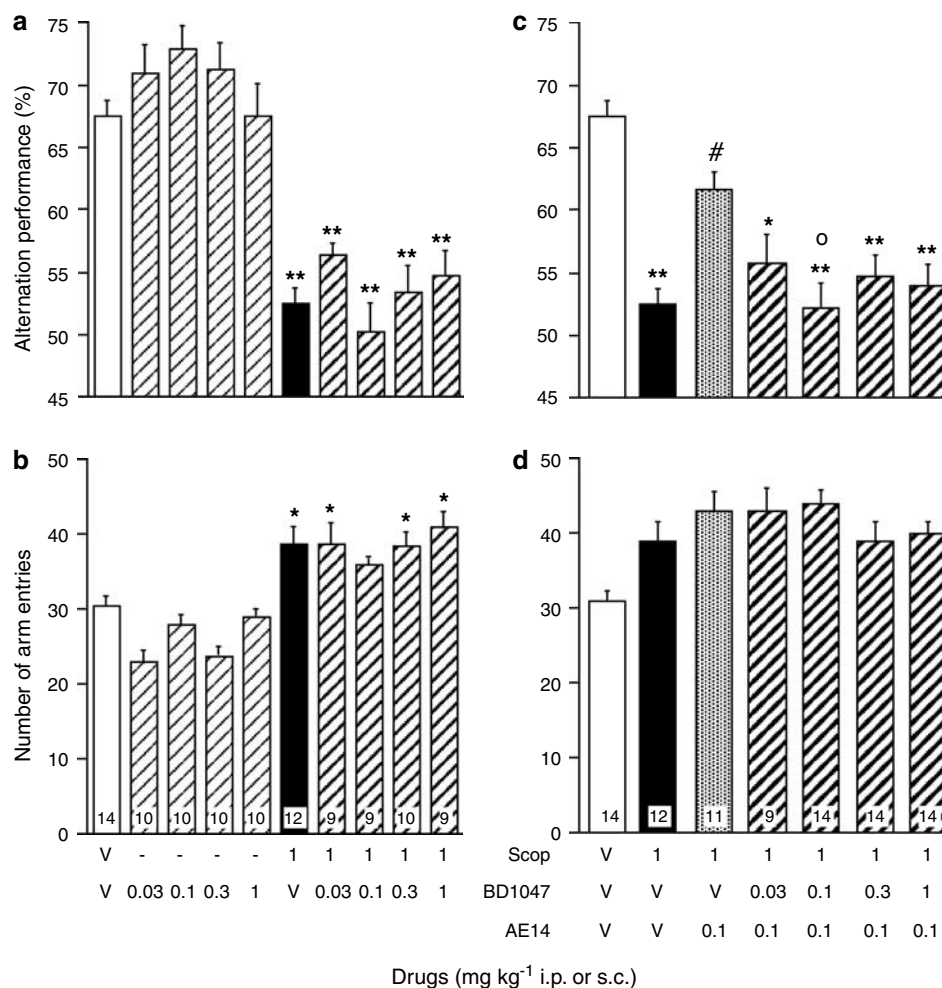


**Figure 4** Effect of ANAVEX1-41 (AE14) on the scopolamine-induced impairment of place learning in the water-maze: (a and b) performances over time, (c) probe test performances and (d) working memory performances. Animals were administered with vehicle solution (Veh, i.p.), ANAVEX1-41 (0.3 mg kg<sup>-1</sup> i.p.) and/or scopolamine (Scop, 1 mg kg<sup>-1</sup> s.c.) 20 min before the first trial and submitted during 5 days to three swims per day. (a and b) Performances over time. Repeated measures ANOVA: Fr = 25.06,  $P < 0.0001$  for the Veh-treated group; Fr = 11.70,  $P < 0.05$  for the Scop-treated group; Fr = 20.69,  $P < 0.001$  for the AE14-treated group; Fr = 27.37,  $P < 0.0001$  for the (AE14 + Scop)-treated group; \* $P < 0.05$ , \*\* $P < 0.01$  vs latencies shown by the Veh-treated group during the same training day; # $P < 0.05$  vs latencies shown by the Scop-treated group; Dunn's test. (c) Probe test. The presence in each quadrant was measured, and presence in the training (T) and opposite (O) quadrants is presented. \*\* $P < 0.01$  vs time spent in the T quadrant for the same experimental group; # $P < 0.05$ , ## $P < 0.01$  vs time spent in the T quadrant for the Veh-treated group; Dunn's test. (d) Working memory procedure. The swimming duration measured for the 1st and 4th swimming trial is presented. \* $P < 0.05$ , \*\* $P < 0.01$  vs trial 1; Dunn's test. ANAVEX1-41, tetrahydro-*N,N*-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride; i.p., intraperitoneally; s.c., subcutaneously; ANOVA, analysis of variance; T quadrant, training quadrant.

retention parameters and, when pre-administered before scopolamine, it failed to affect the scopolamine-induced decreases in latency or percentage of animals-to-criterion (Figures 6a and b). However, when the compound was co-administered with ANAVEX1-41, it dose-dependently blocked the anti-amnesic effect of ANAVEX1-41, with a significant antagonism observed at the dose of 0.3 mg kg<sup>-1</sup> for both latency (Figure 6c) and animals-to-criterion (Figure 6d).

Mice were then treated during 3 days with an antisense ODN (S1R asODN), targeting the  $\sigma_1$  receptor. Control animals received a control mismatch ODN (S1R mODN). After the ODN treatments, mice receiving vehicle or ANAVEX1-41 (0.1 mg kg<sup>-1</sup>) showed an unaltered alternation

performance (Figure 7a). The scopolamine (1 mg kg<sup>-1</sup>) treatment provoked alternation deficits of similar intensity in both groups. Furthermore, the pretreatment with ANAVEX1-41 induced a significant prevention of the scopolamine deficits in both groups (Figure 7a). None of the treatments affected the general exploratory activity in the Y-maze (Figure 7b). However, slightly different results were obtained with the same animals submitted to the passive avoidance procedure. In S1R mODN-treated animals, the scopolamine induced deficits in latency and percentage of animals-to-criterion were significantly prevented by ANAVEX1-41 (Figures 7c and d). On the contrary, the ANAVEX1-41 pretreatment was ineffective in S1R asODN-treated groups (Figures 7c and d), suggesting that the long-term memory



**Figure 5** Blockade by the selective  $\sigma_1$  receptor antagonist BD1047 of the effects of ANAVEX1-41 (AE14;  $0.1 \text{ mg kg}^{-1}$  i.p.) on alternation behaviour in scopolamine-treated mice: (a and c) alternation performances, (b and d) total number of arm entries. (a and b) Lack of effect of BD1047 alone or in combination with scopolamine; (c and d) antagonism studies. BD1047 ( $0.03\text{--}1 \text{ mg kg}^{-1}$  i.p.), ANAVEX1-41 ( $0.1 \text{ mg kg}^{-1}$  i.p.) or vehicle solution (V) was administered 30 min before the test, or 10 min before scopolamine (Scop,  $1 \text{ mg kg}^{-1}$  s.c.), which was administered 20 min before the test. The number of mice per group is indicated in (b and d);  $F(9,92) = 5.68$ ,  $P < 0.0001$  in (a),  $F(9,92) = 4.07$ ,  $P < 0.001$  in (b),  $F(6,77) = 3.18$ ,  $P < 0.01$  in (c) and  $F(6,77) = 1.52$ ,  $P > 0.05$  in (d). \* $P < 0.05$ , \*\* $P < 0.01$  vs the V-treated group; # $P < 0.05$  vs the Scop-treated group; 'o' indicates  $P < 0.05$  vs the (AE14 + Scop)-treated group; Dunnett's test. BD1047, *N*-[2-(3, 4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino) ethylamine hydrochloride; ANAVEX1-41, tetrahydro-*N*,*N*-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride; i.p., intraperitoneally; s.c., subcutaneously.

effect of ANAVEX1-41 preferentially involved an interaction with the  $\sigma_1$  receptor.

#### Involvement of the $\sigma_1$ receptor in the anti-depressant-like effect of ANAVEX1-41

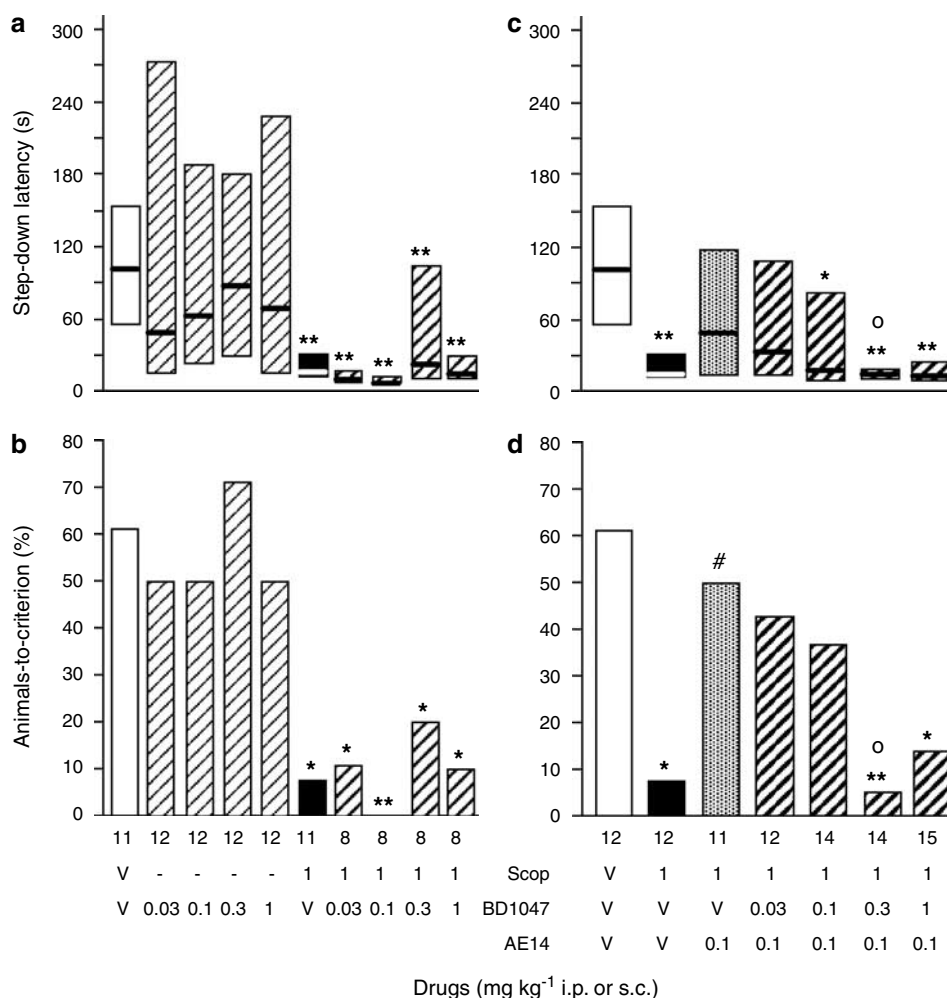
Selective  $\sigma_1$  receptor agonists show marked antidepressant-like activity in the forced swimming test (Matsuno *et al.*, 1996; Urani *et al.*, 2001). Furthermore, an interaction with the  $\sigma_1$  receptor may contribute to the acute antidepressant-like activity of drugs (Villard *et al.*, submitted manuscript). Therefore, we tested ANAVEX1-41 in the forced swimming test, using a larger dose range. The compound significantly shortened the duration of immobility, at 30 and  $60 \text{ mg kg}^{-1}$  (Figure 8a). BD1047, at 4 or  $10 \text{ mg kg}^{-1}$  i.p., failed to affect the immobility duration (Figure 8b). Furthermore, when pre-administered before the active doses of ANAVEX1-41, the  $\sigma_1$  receptor antagonist failed to block the antidepressant effect

of ANAVEX1-41. At  $10 \text{ mg kg}^{-1}$ , for for each dose of ANAVEX1-41, a significant increase in the immobility duration was even observed (Figure 8c). Animals were treated with the S1R asODN or its mODN control, and the antidepressant-like effect of ANAVEX1-41 was examined (Figure 8d). In both groups, ANAVEX1-41 significantly reduced the immobility duration. Moreover, ANAVEX1-41 at  $60 \text{ mg kg}^{-1}$  even tended to be more effective in S1R asODN-treated animals ( $P = 0.3$ ; Figure 8d). It is therefore unlikely that the antidepressant-like activity of ANAVEX1-41 involves an action at the  $\sigma_1$  receptor.

#### Discussion

Selective  $\sigma_1$  receptor agonists have shown effective anti-amnesic, antidepressant and neuroprotective activities in animal models (for reviews, see Maurice *et al.*, 1999, 2001a,



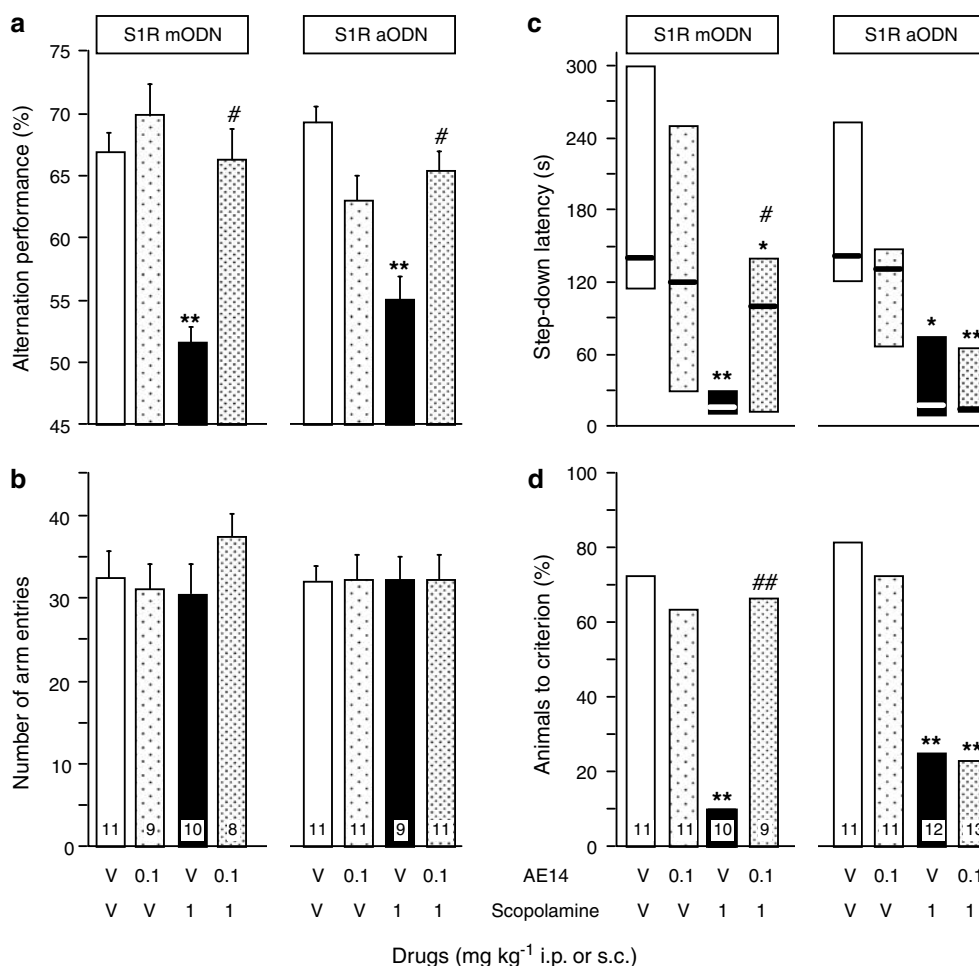


**Figure 6** Blockade by the selective  $\sigma_1$  receptor antagonist BD1047 of the effects of ANAVEX1-41 (AE14; 0.1 mg kg<sup>-1</sup> i.p.) in the step-down passive avoidance test: (a) step-down latency and (b) percentage of animals to criterion. (a and b) Lack of effect of BD1047 alone or in combination with scopolamine; (c and d) antagonism studies. BD1047 (0.03–1 mg kg<sup>-1</sup> i.p.), ANAVEX1-41 (0.1 mg kg<sup>-1</sup> i.p.) or vehicle solution (V) was administered 30 min before the first training, or 10 min before scopolamine (Scop, 1 mg kg<sup>-1</sup> s.c.), which was administered 20 min before the first training. In (a and c), results show the median and interquartile range. The number of mice per group is indicated below the columns in (b and d); KW = 40.64,  $P < 0.0001$  in (a); KW = 21.95,  $P < 0.01$  in (c). \* $P < 0.05$ , \*\* $P < 0.01$  vs the V-treated group; # $P < 0.05$  vs the Scop-treated group; 'o' indicates  $P < 0.05$  vs the (AE14 + Scop)-treated group; Dunn's test in (a and c) and  $\chi^2$ -test in (b and d). BD1047, N-[2-(3, 4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino) ethylamine hydrochloride; ANAVEX1-41, tetrahydro-N,N-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride; i.p., intraperitoneally; s.c., subcutaneously.

2006). At present, no selective compound has yet been marketed for clinical use solely on the basis of its  $\sigma_1$  receptor agonist properties in any therapeutic indication. However, several compounds used against cognitive disorders in humans are known to act, besides their main target, as potent  $\sigma_1$  receptor ligands. This is the case for, among others, donepezil (Aricept), a potent cholinesterase inhibitor used in Alzheimer's disease (Kato *et al.*, 1999; Maurice *et al.*, 2006; Meunier *et al.*, 2006a, b), or sertraline (Zoloft), fluvoxamine (Floxyfral) and opipramol (Insidon), prescribed as antidepressants (Bergeron *et al.*, 1993; Rogoz and Skuza, 2006; Yagasaki *et al.*, 2006). Moreover, several  $\sigma_1$  receptor agonists have been tested in clinical trials for anti-amnesic or antidepressant indications. Igmesine, highly efficient in the forced swimming and tail suspension tests in rodents (Kinsora *et al.*, 1998; Urani *et al.*, 2001), has been tested in clinical trials

with promising results (Pande *et al.*, 1998). Its development is however stopped. The non-selective 5-HT<sub>1A</sub>/ $\sigma_1$  receptor agonist OPC-14523, showing antidepressant properties with a rapid onset of action, is still under development (Tottori *et al.*, 2001; Bermack and Debonnel, 2007).

The mechanism of action of  $\sigma_1$  receptor agonists is at present understood in broad outline. Binding of the ligand to the ER-bound protein induces a rapid facilitation of intracellular calcium fluxes through modulation of inositol-1,4,5 trisphosphate receptor-gated channels (Hayashi *et al.*, 2000). Ca<sup>2+</sup> mobilization leads to the modulation of neurotransmitter responses (Monnet *et al.*, 1992) mainly through facilitation of receptor transduction pathways, activation of intracellular kinases and lipases (Morin-Surun *et al.*, 1999; Nuwayhid and Werling, 2003), and facilitation of neuromodulatory systems, such as those linked to trophic

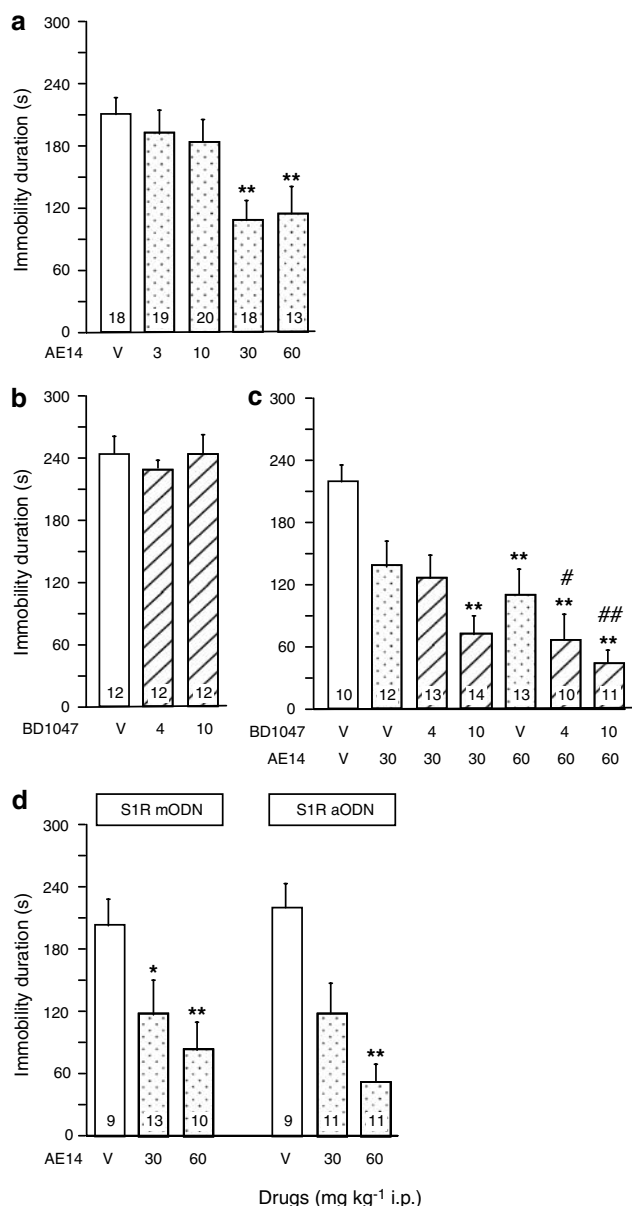


**Figure 7** Effect of the ODN treatments on the anti-amnesic effect of ANAVEX1-41 (AE14) against the scopolamine-induced learning impairments: (a) alternation performance and (b) number of arm entries in the Y-maze, (b) latencies and (c) percentage of animals to criterion during retention in the passive avoidance test. The  $\sigma_1$  receptor antisense ODN (S1R asODN) or mismatch control ODN (S1R mODN) were administered i.c.v. every 12 h during 3 days. ANAVEX1-41 (0.1 mg kg<sup>-1</sup> i.p.) was administered 10 min before scopolamine (1 mg kg<sup>-1</sup> s.c.), which was given 20 min before the Y-maze session or the first passive avoidance training session. The number of mice per group is indicated in (b and d);  $F(3,72)=7.19$ ,  $P<0.001$  for the treatment,  $F(1,72)=0.04$ ,  $P>0.05$  for the ODN and  $F(3,72)=0.90$ ,  $P>0.05$  for the treatment  $\times$  ODN interaction in (a);  $F(3,72)=0.55$ ,  $P>0.05$  for the treatment,  $F(1,72)=0.10$ ,  $P>0.05$  for the ODN and  $F(3,72)=0.53$ ,  $P>0.05$  for the treatment  $\times$  ODN interaction in (b); and  $KW=11.32$ ,  $P<0.05$  for S1R mODN and  $KW=14.38$ ,  $P<0.01$  for S1R asODN in (c). \* $P<0.05$ , \*\* $P<0.01$  vs the (V + V)-treated group; # $P<0.05$ , ## $P<0.01$  vs the (V + Scop)-treated group; Dunnett's test in (a), Dunn's test in (c),  $\chi^2$ -test in (d). ODN, oligodeoxynucleotide; ANAVEX1-41, tetrahydro-*N,N*-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride; i.c.v., intracerebroventricularly; i.p., intraperitoneally.

factors (Yagasaki *et al.*, 2006). Activation of  $\sigma_1$  receptors also leads to the translocation of the protein towards the plasma membrane, where it promotes the formation and recomposition of caveolin-2-labeled lipid microdomains, resulting in brain plasticity changes (Hayashi and Su, 2003a,b). Under chronic activation,  $\sigma_1$  receptors indeed mediate important morphologic changes in neurons, promoting neurite outgrowth and sprouting, and neurogenesis (Takebayashi *et al.*, 2004). These effects support a role for  $\sigma_1$  receptors in learning and memory, depression and neuroprotection.

The pharmacological profile of ANAVEX1-41 is therefore particularly interesting, since the compound is a mixed muscarinic and  $\sigma_1$  receptor ligand, equipotent on M<sub>1</sub>/M<sub>3</sub>/M<sub>4</sub> and  $\sigma_1$  sites. Analysis of the anti-amnesic properties of the compound showed that it blocked scopolamine-induced amnesia. The compound was effective in the spontaneous

alternation, passive avoidance and place learning test in the water-maze, using either the reference or working memory procedure. The compound appeared to be equally effective in reversing amnesia affecting short- and long-term, spatial and contextual memory processes. At the pharmacological level, both the muscarinic and  $\sigma_1$  components are involved in ANAVEX1-41 action. Moreover, when the  $\sigma_1$  receptor expression in the hippocampus and cortex was down-regulated using the *in vivo* repeated antisense ODN treatment, ANAVEX1-41 remained effective in reversing the scopolamine-induced spontaneous alternation deficits. This observation suggests that the muscarinic component of ANAVEX1-41 pharmacological action is sufficient to maintain the short-term memory enhancing properties of the compound when the  $\sigma_1$  receptor expression is down-regulated. Indeed, working memory is highly dependent



**Figure 8** Antidepressant-like effect of ANAVEX1-41 (AE14) in the forced swimming test. The dose-response effect of ANAVEX1-41 (a), of BD1047 alone (b), the combination of ANAVEX1-41 with BD1047 (c), and the effects of ODN treatment on the responses to AE-14 (d). Drugs were injected i.p. 30 min before the test session on day 2. The duration of immobility was recorded during the last 5 min over a 6-min session. In (d), ODN was administered i.c.v. twice a day during 3 days. The pre-test session was performed on day 3 and drugs were administered i.p. on day 4, 30 min before the test session. The number of mice per group is indicated within the columns;  $F(4,64) = 3.22$ ,  $P < 0.05$  in (a);  $F(2,18) = 0.25$ ,  $P > 0.05$  in (b);  $F(6,72) = 7.85$ ,  $P < 0.0001$  in (c);  $F(2,57) = 13.98$ ,  $P < 0.0001$  for the dose,  $F(1,57) = 0.05$ ,  $P > 0.05$  for the ODN and  $F(2,57) = 0.40$ ,  $P > 0.05$  for the dose  $\times$  ODN interaction in (d). \* $P < 0.05$ , \*\* $P < 0.01$  vs the V-treated group; # $P < 0.05$ , ## $P < 0.01$  vs the AE14-treated group; Dunnett's test. ANAVEX1-41, tetrahydro-*N,N*-dimethyl-5,5-diphenyl-3-furanmethanamine hydrochloride; BD1047, *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(dimethylamino) ethylamine hydrochloride; i.p., intraperitoneally; ODN, oligodeoxynucleotide; i.c.v., intracerebroventricular.

on muscarinic cholinergic systems. For instance, Moran (1993) by examining the scopolamine vs mecamylamine effect on T-maze alternation and discrimination tasks in rats observed that working memory tasks are more sensitive to central muscarinic blockade than reference memory tasks. Ohno *et al.* (1994) observed a clear involvement of hippocampal muscarinic receptors in working memory but not in reference memory, in rats tested using the three-panel runway task.

On the other hand, selective  $\sigma_1$  receptor agonists are known to alleviate scopolamine-induced amnesia. Selective agonists such as igmesine, PRE-084 or SA4503 not only prevented the scopolamine-induced learning impairments, when injected before scopolamine, but also reversed the deficits, when injected before retention (Earley *et al.*, 1991; Matsuno *et al.*, 1995, 1997; Senda *et al.*, 1996, 1997; Maurice *et al.*, 1999). The mode of action of  $\sigma_1$  receptor ligands on cholinergic neurons may involve several pathways. First, activation of the  $\sigma_1$  receptor directly increases acetylcholine release, as measured in the rat hippocampus and cortex using *in vivo* microdialysis (Kobayashi *et al.*, 1996). Second, activation of the  $\sigma_1$  receptor modulates *N*-methyl-D-aspartate receptor-mediated neuronal activity in the hippocampus and recruits  $\text{Ca}^{2+}$ -dependent second messenger cascades, involving protein kinase C, in hippocampal neurons (Monnet *et al.*, 1992, 2003), through a mechanism that is likely to be involved post-synaptically in cholinergic neurons. In the present study, an acute pretreatment with the  $\sigma_1$  receptor antagonist BD1047 blocked the anti-amnesic effect of ANAVEX1-41 in the Y-maze and passive avoidance test, showing that the acute pharmacological effects of ANAVEX1-41 were dependent on  $\sigma_1$  receptors. Interestingly, the repeated antisense ODN treatment blocked the anti-amnesic effect of ANAVEX1-41 in the passive avoidance test, suggesting that ANAVEX1-41 is more effective on long-term memory processes through the involvement of its  $\sigma_1$  receptor component. Moreover, since the lowest anti-amnesic dose ( $0.1 \text{ mg kg}^{-1}$  i.p.) is far below doses inducing cholinergic or  $\sigma_1$  receptor-related side-effects and its putative agonist activity at  $\text{M}_1$  muscarinic receptors (Vamvakides, 2002), ANAVEX1-41 may act on the highly sensitive extrasynaptic muscarinic receptors (Vizi *et al.*, 2004; Vizi and Mike, 2006), by blockade of  $\text{M}_2$  autoreceptors, and on presynaptic  $\text{M}_3$  receptors, present on glutamatergic terminals in the hippocampus and cortex (Sugita *et al.*, 1991; Caulfield, 1993; Stillman *et al.*, 1993). These effects may happen in synergy with the action at  $\sigma_1$  receptors and explain the resistance of the short-term anti-amnesic properties of ANAVEX1-41 after  $\sigma_1$  antisense ODN treatment.

The last part of the study examined if the antidepressant properties of ANAVEX1-41 (Vamvakides, 2002) involved an interaction with the  $\sigma_1$  receptor, as previously observed for selective  $\sigma_1$  receptor ligands (Matsuno *et al.*, 1996; Urani *et al.*, 2001) or antidepressants. The compound reduced the immobility duration in the forced swim test, at the doses of 30 and  $60 \text{ mg kg}^{-1}$  i.p. A shift in active dose between anti-amnesic effects ( $< 1 \text{ mg kg}^{-1}$ ) and antidepressant effects ( $20\text{--}60 \text{ mg kg}^{-1}$ ) has been observed for all  $\sigma_1$  receptor ligands. Progesterone is massively released in target structures like the hippocampus, during acute stress, and the steroid acts as a

potent  $\sigma_1$  receptor antagonist (Urani *et al.*, 2001). The  $\sigma_1$  receptor agonists must therefore compete with the steroid to induce their pharmacological effect. The shift in active dose observed for ANAVEX1-41, 0.1 mg kg<sup>-1</sup> for the reversion of scopolamine-induced amnesia vs 30–60 mg kg<sup>-1</sup> for the reduction of forced swimming-induced immobility, was in line with its  $\sigma_1$  receptor activity. However, neither a pretreatment with the  $\sigma_1$  receptor antagonist BD1047 nor a repeated pretreatment with the antisense ODN affected the antidepressant-like effect of ANAVEX1-41, at both doses tested. Activation of the  $\sigma_1$  receptor is therefore not involved in the antidepressant effect of ANAVEX1-41. Thus, as previously discussed (Vamvakides, 2002), inhibition of sodium channels by ANAVEX1-41 seems to be the most probable candidate for its anti-immobility effect, in this context. However, the exact mechanism of the putative antidepressant action of ANAVEX1-41 deserves a precise study.

In conclusion, the aminotetrahydrofuran derivative ANAVEX1-41 shows a particular anti-amnesic efficacy against scopolamine-induced learning impairments, measured using several behavioural procedures assessing short- and long-term memory tests, and involving a mixed activity at muscarinic and  $\sigma_1$  receptors. Its antidepressant-like activity seems to selectively involve its inhibitory activity on sodium channels. However, the mixed muscarinic/ $\sigma_1$  receptor pharmacological profile may be of particular promise in developing new drugs for neurodegenerative diseases.

## Acknowledgements

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## Conflict of interest

These authors state no conflict of interest.

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