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ERK1/2 phosphorylation is involved in the antidepressant-like action of 2,5-diphenyl-3-(4-fluorophenylseleno)-selenophene in mice

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ABSTRACT

We investigated the antidepressant-like action of 5 compounds belonging to the selenophene class. The involvement of ERK and CREB activation in this action was also demonstrated. In the experiment 1, time-course and dose-response effect of **H-DPS**, **CH_3-DPS**, **Cl-DPS**, **F-DPS** and **CF_3-DPS** were accompanied in the mouse forced swimming test (FST). Firstly, animals received compounds at a dose of 50 mg/kg, by intragastric (i.g.) route, at different times (15–240 min) before test. Results showed that the peak of maximum anti-despair behavior induced by **Cl-DPS**, **F-DPS** and **CF_3-DPS** was at 30 min; maximum effect of **H-DPS** and **CH_3-DPS** was found at 60 min, which was maintained until 120 min. Regarding dose–response effect, all compounds reduced immobility time and increased latency for the first episode of immobility at a dose of 50 mg/kg. In addition, **F-DPS** also showed antidepressant-like action at a dose of 25 mg/kg, whilst **H-DPS**, **CH_3-DPS**, **Cl-DPS** and **CF_3-DPS** were not effective at lower doses. Thus, **F-DPS** was chosen for further investigation of its mechanism of action. Experiment 2 showed that treatment of animals with **F-DPS** (50 mg/kg, i.g.) significantly increased phosphorylated ERK1/2 levels in the prefrontal cortex and hippocampus; however, pCREB levels were not affected. Additionally, in the experiment 3 anti-immobility effect of **F-DPS** was completely blocked by pretreatment of animals with PD 98,059, an inhibitor of ERK phosphorylation, suggesting that ERK signalling activation is involved in its antidepressant-like action in mice. Together our data appoint **F-DPS** as a promising molecule for the development of a new antidepressant therapy.

1. Introduction

Organic forms of selenium have been suggested as relevant biologic agents (Nogueira and Rocha, 2011). Over the last decade preclinical studies have demonstrated that several organoselenium compounds have pharmacological properties including antioxidant, anticonvulsant, neuroprotective and antidepressant actions (Bruning et al., 2011; Dias et al., 2014; Gai et al., 2013; Mahadevan et al., 2013; Pinton et al., 2013; Wilhelm et al., 2012).

We highlight the selenophene class, a specific group of heterocyclic selenium-containing molecules whose members have been shown to produce antidepressant-like properties (Gai et al., 2014, 2013, 2012; Gay et al., 2010). A recent study showed that the administration of a single dose of 3-chalcogen selenophenes (50 mg/kg) reduced the immobility time in the mouse forced swimming test (FST); structure–activity relationship studies demonstrated that the phenyl group at the 2-position and an organoselenium group at the 3-position of the five-member ring are essential for their antidepressant-like activity (Gai et al., 2012). However, although the antidepressant-like action selenophene compounds is known, the pharmacological profile of 3-chalcogen selenophenes was not further investigated. Furthermore, mechanisms involved in their antidepressant-like action remain still unclear.

Although most studies focus on serotonergic and noradrenergic systems (Millan, 2004), recent evidences have identified modifications of intracellular signalling proteins and target genes that could contribute to the pharmacological action of antidepressant therapy (Blendy, 2006; Carreno and Frazer, 2014; Kuo et al., 2013; Reus et al., 2011). Modulation of mitogen-activated protein kinase (MAPK) pathways and the transcription factor CREB (cAMP-response element-binding protein) are molecular targets for antidepressants (Blendy, 2006; Kuo et al., 2013). CREB is upregulated by antidepressant treatment, and increasing CREB levels in rodent

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models results in antidepressant-like behaviors (Blendy, 2006; Carreno and Frazer, 2014; Reus et al., 2011). Phosphorylation, and therefore activation of CREB can be induced by a number of upstream signalling cascades, including MAPK/ERK (Blendy, 2006). The mitogen activated protein kinase (MAPK) pathway is a major signalling system that regulates cellular responses and activation of the MAPK cascade plays a critical role in the pathophysiology of depression (Roux and Blenis, 2004). It has been shown that inhibition of MAPK signalling produces a depressive-like phenotype and blocks behavioral actions of antidepressants. Extracellular signal-regulated protein kinases (ERKs) are MAPKs that are involved in cell proliferation and neuroprotection (Mebratu and Tesfaigzi, 2009). Although the role of ERK in depression is unclear, previous reports showing decreased phosphorylated levels of ERK1/2 in the post-mortem brain of depressed suicide subjects and in rodent models of depression suggest an important involvement of this protein in mood disorders (Dwivedi et al., 2001; Feng et al., 2003). Furthermore, recent studies have demonstrated a positive modulation of ERK isoforms by antidepressant drugs such as fluoxetine and reboxetine (First et al., 2013; Kuo et al., 2013).

Taking into account the above mentioned points, the main objective of this study was to demonstrate the profile of antidepressant-like action of selenophene compounds in mice as well as to investigate the participation of ERK and CREB phosphorylation in this effect.

2. Materials and methods

2.1. Animals

The experiments were conducted using male Swiss mice (25–30 g) maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out between 08.00 a.m. and 04.00 p.m and mice were acclimated to the behavioral room at least 2 h before the test. The experiments were performed according to a randomized schedule and each animal was used only once in each test. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil (# 124/2010). The procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimise animals suffering and to reduce the number of animals used in the experiments.

2.2. Drugs

2,5-Diphenyl-3-(phenylseleno)-selenophene **(H-DPS)**, 2,5-diphenyl-3-(4-methylphenylseleno)-selenophene **(CH_3-DPS)**, 2,5-diphenyl-3-(4-chlorophenylseleno)-selenophene **(Cl-DPS)**, 2,5-diphenyl-3-(4-fluorophenylseleno)-selenophene **(F-DPS)** and 2,5-diphenyl-3-(3-trifluoromethylphenylseleno)-selenophene **(CF_3-DPS)** (Fig. 1), were prepared and characterised in our laboratory by the method previously described (Stein et al., 2008). Analysis of the ^{1}HNMR and ^{13}CNMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of studied compound (99.9%) was determined by GC/MS.

Specific antibody against ERK1/2 phosphorylated (pERK1/2) was obtained from Cell Signalling Technology®. Antibody against CREB phosphorylated on Ser133 (pCREB) and β-actin were obtained from Santa Cruz Biothechnology®. PD98,059 was purchased from Sigma®. All other chemicals were obtained from analytical grade and standard commercial suppliers.

2.3. Experimental design

2.3.1. Experiment 1

This experiment was performed in order to analyse the pharmacological profile of antidepressant-like action of selenophene compounds (**H-DPS**, **CH_3-DPS**, **Cl-DPS**, **F-DPS** and **CF_3-DPS**) in the mouse forced swimming test (FST).

To assess the time-course of antidepressant-like action of selenophene compounds, separate groups of mice received a single administration of canola oil (10 ml/kg) or **H-DPS**, **CH_3-DPS**, **Cl-DPS**, **F-DPS** and **CF_3-DPS** at a dose of 50 mg/kg, administered by intragastric (i.g.) route. At different times after treatment (15, 30, 60, 120, 180 or 240 min) animals were evaluated in the FST (n=6–8 animals/group). Pretreatment time in which compounds presented maximum anti-immobility effect in the FST was chosen for performing the subsequent step (Fig. 1).

To obtain the dose–response assessment**,** compounds were administered at doses of 10, 25 and 50 mg/kg, i.g., 30 min (**Cl-DPS**, **F-DPS** and **CF_3-DPS**) or 60 min (**H-DPS** and **CH_3-DPS**) before FST (n=6–8 animals/group). Animals were also evaluated in the locomotor activity monitor (LMA) in order to rule out any interference of locomotion in the FST (Fig. 1).

Since **F-DPS** produced antidepressant-like action at lower doses than other organoselenium compounds, it was chosen for performing experiments 2 and 3.

2.3.2. Experiment 2

To investigate whether ERK1/2 phosphorylation is modulated by **F-DPS**, firstly mice received a single i.g. dose of **F-DPS** 50 mg/kg or canola oil (10 ml/kg; n=4/group). Thirty min after treatment animals were then killed by decapitation and hippocampi and prefrontal cortices were removed for determination of pERK1/2 (Fig. 1). Since MAP kinases, like ERK, regulate the activities of several transcription factors, such as CREB, pCREB expression was also determined. Because total protein levels are generally no modulated after a short period, only phosphorylated proteins were quantified.

Additionally, since single treatment did not modify pCREB levels, we performed a repeated treatment with **F-DPS** in order to analyse this protein. Compound was administered at a dose of 50 mg/kg, i.g, during 3 consecutive days and hippocampus and prefrontal cortex of mice were removed 30 min after the last dose for pCREB expression (Fig. 1).

In a separate group of animals, **F-DPS** (50 mg/kg, i.g.) or canola oil (10 ml/kg; n=4/group) were administered to mice. After 30 min, mice were anaesthetised and brains were perfused with paraformaldehyde solution for pERK1/2 staining (Fig. 1).

2.3.3. Experiment 3

In order to assess the antidepressant-like action of **F-DPS** in the tail suspension test (TST), mice were previously treated with canola oil (10 ml/kg, n=6–8) or F-DPS at doses of 10, 25 and 50 mg/kg (i.g.; n=6–8) and subjected to the TST after 30 min.

To test the hypothesis that the antidepressant-like action of F-DPS is mediated through a modulation of ERK phosphorylation, a separated group of animals was treated with saline (5 μl/site) or PD98,059 (a MEK inhibitor, 20 μg/site), by i.c.v. route, one hour before the canola oil or F-DPS administration (50 mg/kg. i.g.; n=6/group). The TST was carried out 30 min after F-DPS treatment (Fig. 1).

2.4. Spontaneous locomotor activity

To discard non-specific effects of treatments, spontaneous locomotor activity of mice was performed in the locomotor activity monitor (LMA). LMA is a Plexiglas cage (45 × 45 × 45 cm^3) surrounded

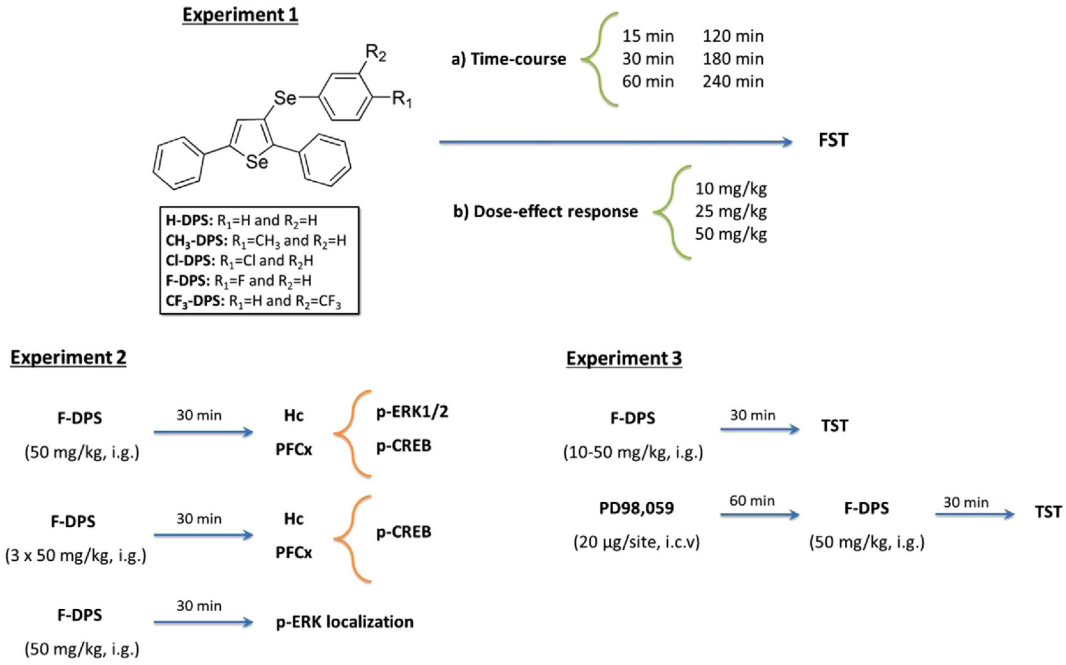

Fig. 1. Schematic representation of the experimental design of this study.

by a frame consisting of 32 photocells mounted on opposite walls (16 L × 16 W, spaced 2 cm apart) that continuously tracks the animal's movement. After a previous habituation during 1 min, animals were placed in the center of the apparatus and allowed to freely explore the arena during 4 min. Motor activity was monitored with the Insight® Monitor Activity System. Data were collected in the form of photobeam breaks as an indication of activity within different predetermined "zones" in the open field using Monitor Activity® software (Insight). Number of crossings and rearings, average velocity (mm/s) and total distance traveled (dm) were recorded.

2.5. FST

The FST is one of the most widely used tools for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at rendering or preventing depressive-like states. The procedure used in this study was based on that previously described (Porsolt et al., 1979). Mice were gently placed in an inescapable cylindrical container ($10 \times 25\ cm^2$) that was filled with water (19 cm, 25 ± 1 °C) and their escape related mobility behavior (latency for the first immobility episode and total duration of floating) was measured by a blinded observer during a 6 min period by using a stopwatch. Latency was defined as the amount of time that elapsed between placing the mouse in the tank and the first instance of each behavioral occurrence. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water.

2.6. TST

The TST was performed in a quiet experimental room according to the method reported by Steru and collaborators (Steru et al., 1985). Each mouse was suspended by its tail to an horizontal wooden bar located inside a yellow plastic box (40 cm × 46 cm × 40 cm) approximately 30 cm above the floor. The mouse was secured to the bar by adhesive tape placed 1 cm from the tip of the tail, such that the mouse's head was about 20 cm above the floor. The trial was conducted for 6 min during which a blinded observer scored the latency for the first immobility episode and total duration of immobility by using a stopwatch. The mouse was considered immobile only when it hung passively and completely motionless. Mice that climbed their tails were eliminated from further analyses.

2.7. Intracerebroventricular (i.c.v.) injection

The i.c.v. injection was carried out as previously described (Haley and McCormick, 1957) and modified (Laursen and Belknap, 1986), with the bregma fissure as a reference point. A 0.4 mm external diameter hypodermic needle was briefly attached to a cannula, which was linked to a 25 µl Hamilton syringe, inserted perpendicularly through the skull no more than 2 mm into the brain of the mouse. A volume of 5 µl was then administered in the left lateral ventricle. The injection was given over 30 s, and the needle remained in place for another 30 s in order to avoid the reflux of the substances injected. The injection site was 1 mm to the right or left from the mid-point on a line drawn through to the anterior base of the ears. In order to avoid interference in the behavioral testing, no anesthetic drug was administered. Animals were immobilised during the injection.

2.8. Western blot analysis

After **F-DPS** treatment, animals were killed by decapitation, brains were removed and prefrontal cortex and hippocampus were dissected in a near-freezing environmental to avoid the rapid post-mortem degradation of phosphoproteins. The hippocampi and prefrontal cortices were homogenised in ice-cold ysis buffer containing 25 mM TrisHCl (pH 7.5), 25 mM NaCl, 5 mM EGTA, 2.5 mM EDTA, 2 mM NaPP, 4 mM PNFF, 1 mM Na_3VO_4, 1 mM PMSF, 20 µg/ml leupeptin, 50 µg/ml aprotinin, 0.1 % SDS. The homogenate was centrifuged at 9000 × g for 20 min at 4 °C, the low speed pellet was discarded. Samples were stored at −80 °C till analysis. The supernatant (whole cell lysate) was separated on 10 % SDS-PAGE and transferred onto nitrocellulose membranes (120 min at 100 V) using standard procedures. Membranes were blocked in PBST (PBS containing 0.1 % Tween) containing 5% nonfat dry milk for 120 min. Following washes, blots were incubated

overnight at 4 °C with specific antibodies against pERK1/2 (1:1000), pCREB (1:500) and β-actin (1:3000 dilution). After being washed with PBS containing 0.1% Tween, the nitrocellulose membrane was incubated with a horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (1:5000) and left for 2 h at room temperature. Blots were then extensively washed according to the manufacturer's instruction and developed using enhanced chemiluminescence detection system (Pierce, Milan, Italy). Exposition and developing time used was standardized for all the blots. Optical density measurements were performed by dividing the intensity of the bands by the intensity of the housekeeping protein β-actin, used as loading control, at each time point. Measurements in control samples were assigned a relative value of 100%.

2.9. Protein determination

Protein concentration was measured by biuret method using bovine serum albumin (1 mg/ml) as the standard (Gornall et al., 1949).

2.10. Tissue preparation and immunofluorescence

After **F-DPS** treatment mice were anaesthetised with 4% chloral hydrate and perfused through the left cardiac ventricle with 10 ml of a cold fixative (4% paraformaldehyde in 100 mM phosphate buffer). After perfusion, animals were subjected to decapitation and the brain tissues were quickly removed, postfixed for 18 h with the same fixative at 4 °C, and transferred to 10%, then 20%, and then 30% sucrose solution. Brains were cut on a cryostat and sections (40 μM) were stored at −80 °C in an anti-freeze cryoprotectant solution (28% glycerol/28% ethylene glycol) till analysis. After preincubation in 5 mg/ml BSA/0.3% Triton-X-100/PBS, sections were incubated overnight at 4 °C with primary antibody at optimised working dilution. pERK antibody (1:50, SantaCruz Biothechnology Inc, CA, USA) was detected by Alexa 488-conjugated rabbit secondary antibody (1:200; Invitrogen, Carlsbad, CA). Sections were coverslipped using Vectorshield mounting medium with DAPI (Vector Laboratories, Burlingame, CA). A Leica DF 350 FX microscope with appropriate excitation and emission filters for each fluorophore was used to acquire representative images. Images were acquired with 20× to 40× objectives using a digital camera.

2.11. Statistical analysis

All experimental results are given as the mean ± S.E.M. First, we evaluated the normality of data using the D'Agostino and Pearson omnibus normality test. Comparisons between experimental and control groups in Experiments 1 (FST and LMA) and 3 (TST) were performed by one-way analysis of variance (ANOVA) followed by Newman–Keuls test for post hoc comparison when appropriate. Results from pERK1/2 and pCREB levels (Experiment 2) were analyzed by using unpaired *t*-test. Blocking effect of PD98,059 on the antidepressant-like action of F-DPS in the TST was analyzed by two-way ANOVA followed by Newman–Keuls test. Main effects of first order interactions are presented only when interaction was not significant. All analyses were performed using the STATISTICA for Windows software Version 7 (StatSoft, Oklahoma, USA). Probability values less than 0.05 ($P<0.05$) were considered as statistically significant.

3. Results

3.1. Selenophene compounds produce antidepressant-like action in the FST without any change in locomotor activity of animals

Our results showed that all selenophene compounds tested in this study showed antidepressant-like actions in the mouse FST. However, the pharmacological profile (dose and pretreatment time) was different for each drug.

As shown in Figs. 2A and B, acute treatment with **H-DPS** (50 mg/kg, i.g.) significantly modified total immobility time [$F_{(5,34)}=15.49$, $P<0.001$; Fig. 2A] and latency for the first episode of immobility in the FST [$F_{(5,34)}=2.56$, $P<0.05$; Fig. 2B]. Post hoc analyzes showed that **H-DPS** decreased the immobility duration at 60 (64.3%, $P<0.001$) and 120 (53.8%, $P<0.001$) min after a single administration. Latency was increased at 60 min after treatment (47.2%, $P<0.01$). Treatment of animals with **H-DPS** at doses of 10 and 25 mg/kg, 60 min before FST, did not produce any significant difference in the immobility time and latency for the first episode of immobility when compared to the control group ($P>0.05$; Table 1).

In a similar way to **H-DPS**, treatment of mice with **CH$_3$-DPS**, at a dose of 50 mg/kg, produced a significant effect on immobility time [$F_{(5,36)}=7.40$, $P<0.001$; Fig. 2C] and latency in the FST [$F_{(5,36)}=3.01$, $P<0.05$; Fig. 2D]. Total immobility time of mice was decreased around of 30% ($P<0.01$) after 60 and 120 min of **CH$_3$-DPS** treatment whilst latency for the first episode was increased around of 57.5% ($P<0.05$) at the pretreatment time of 60 min when compared to the vehicle-treated group. Doses of 10 and 25 mg/kg, 60 min before FST, did not produce significant effect ($P>0.05$; Table 1).

Treatment of animals with **Cl-DPS** produced the longest antidepressant-like action when compared with other selenophene compounds (Fig. 2). Latency [$F_{(6,48)}=6.56$, $P<0.001$; Fig. 2E] and immobility duration [$F_{(6,47)}=5.09$, $P<0.001$; Fig. 2F] were significantly modified after a single dose of 50 mg/kg **Cl-DPS**. Post hoc analyzes showed that **Cl-DPS** decreased immobility at pretreatment times of 30 min (22.6%, $P<0.05$), 60 min (31.4%, $P<0.05$;), 120 min (34.5%, $P<0.01$) and 180 min (32.7%, $P<0.01$). Latency for the first episode of immobility was increased around of 47.6% ($P<0.01$; 30 min), 50.5% ($P<0.001$; 60 min), 36.9% ($P<0.05$; 120 min) and 35.9% ($P<0.05$; 180 min) after single treatment with **Cl-DPS**. Administration of **Cl-DPS** at doses of 10 and 25 mg/kg, 60 min before FST, did not produce significant antidepressant-like action ($P>0.05$; Table 1).

Figs. 2G and H shows the pharmacological profile of **F-DPS** in the FST. One-way ANOVA yielded a significant effect of **F-DPS** on total immobility time [$F_{(3,23)}=17.97$, $P<0.001$; Fig. 2G] and latency for the first episode of immobility [$F_{(3,23)}=6.86$, $P<0.01$; Fig. 2H]. At 30 min after a single dose of 50 mg/kg, animals treated with **F-DPS** showed a decrease in the total immobility duration (47.5%, $P<0.001$) and an increase in latency (103.2%, $P<0.01$). Moreover, whilst a dose of 10 mg/kg was not effective in the FST ($P>0.05$), administration of **F-DPS** at a dose of 25 mg/kg produced a significant antidepressant-like action in the FST: immobility time was decreased at around 67.3% and latency was increased at approximately 53% when compared to the control group treated with vehicle (Table 1).

Finally, Figs. 2I and J shows the antidepressant-like action of **CF$_3$-DPS**. Similar to the other selenophene compounds, our results demonstrated that acute treatment with **CF$_3$-DPS** produced a significant anti-immobility effect in the FST [$F_{(4,29)}=4.50$, $P<0.01$; Fig. 2I]. Latency for the first episode of immobility was also modified by treatment with this organoselenium compound [$F_{(4,29)}=2.28$, $P<0.05$; Fig. 2J]. Post hoc comparison showed that at 30 min after **CF$_3$-DPS** treatment (50 mg/kg) immobility duration

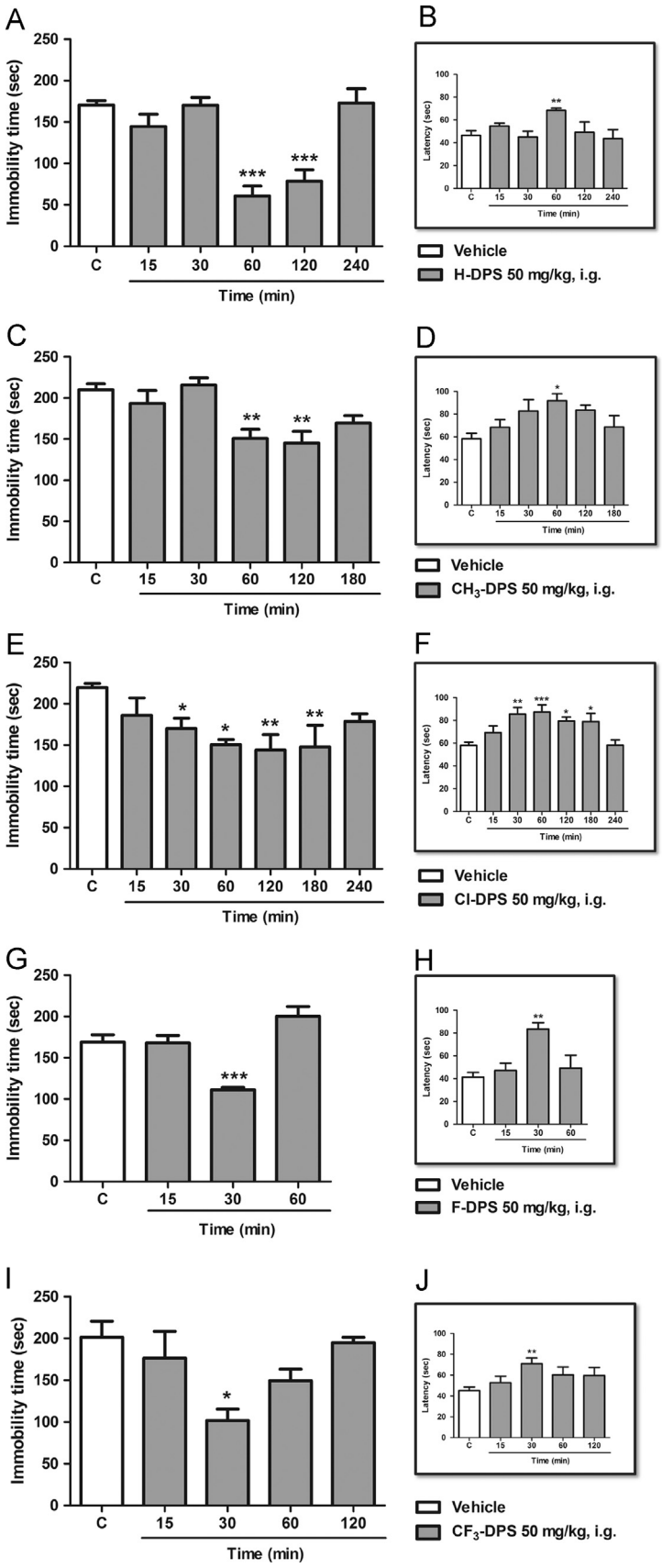

Fig. 2. Effect of acute treatment with selenophene compounds on depression-related behavior in the FST. (A), (C), (E), (G) and (I) represent the total immobility time; (B), (D), (F), (H) and (J) represent the latency to the first episode of immobility. **H-DPS** (A and B), **CH_3-DPS** (C and D), **Cl-DPS** (E and F), **F-DPS** (G and H) and **CF_3-DPS** (I and J) were administered to mice at a dose of 50 mg/kg, i.g. Animals performed FST at different times (15–240 min) after treatment. Values are expressed as mean ± S.E.M. of 6–8 animals. Data were analyzed by using a one-way analysis of variance (ANOVA), followed by the Newman–Keuls test. Asterisks denote the significance level when compared to the control group treated with vehicle: (*) $P < 0.05$, (**) $P < 0.01$ and (***) $P < 0.001$.

Table 1
Effect of acute treatment with selenophene compounds (dose-range: 10–50 mg/kg) on depression-related behavior in the mouse forced swimming test (FST).

	Latency (s)	Immobility time (s)
H-DPS (mg/kg, i.g., 60 min)		
0	47.8 ± 3.5	170.4 ± 5.6
10	51.5 ± 3.6	167.5 ± 12.7
25	52.8 ± 4.5	174.5 ± 24.6
50	68.3 ± 2.0[b]	60.8 ± 12.0[c]
CH_3-DPS (mg/kg, i.g., 60 min)		
0	55.5 ± 3.8	221.2 ± 8.0
10	56.3 ± 3.9	197.5 ± 11.8
25	69.7 ± 6.3	181.2 ± 12.5
50	90.0 ± 7.9[b]	135.7 ± 15.0[c]
Cl-DPS (mg/kg, i.g., 30 min)		
0	58.0 ± 5.5	210.0 ± 6.0
10	61.3 ± 5.3	164.7 ± 26.6
25	72.8 ± 8.0	174.7 ± 16.7
50	88.8 ± 6.8[a]	107.2 ± 14.0[b]
F-DPS (mg/kg, i.g., 30 min)		
0	50.5 ± 4.7	163.2 ± 8.0
10	50.0 ± 5.6	145.2 ± 24.4
25	77.2 ± 8.1[a]	53.3 ± 4.6[c]
50	80.0 ± 5.0[a]	54.8 ± 7.9[c]
CF_3-DPS (mg/kg, i.g., 30 min)		
0	51.3 ± 4.4	201.3 ± 19.4
10	57.6 ± 5.1	172.5 ± 11.0
25	51.8 ± 2.5	191.2 ± 13.3
50	75.5 ± 5.2[b]	101.8 ± 13.8[b]

Selenophene compounds were administered by intragastric (i.g.) route 30 or 60 min before evaluation in the FST. Values are expressed as mean ± S.E.M of 6–8 animals/group. Data were analysed by using a one-way analysis of variance (ANOVA), followed by Newman–Keuls test. Asterisks denote the significance levels when compared to the vehicle group.

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

of mice in the FST was significantly decreased (50.6%, $P < 0.05$) whilst latency was increased (57.1%, $P < 0.01$). Despair parameters in the FST were not modified by lower doses of **CF_3-DPS** (10 and 25 mg/kg, $P > 0.05$; Table 1).

Regarding locomotor activity of mice acutely treated with selenophene compounds, statistical analysis showed that neither the number of crossings and rearings, velocity nor total distance travelled were modified by **H-DPS, CH_3-DPS, Cl-DPS, F-DPS** or **CF_3-DPS** at doses of 10, 25 and 50 mg/kg ($P > 0.05$; Table 2).

3.2. F-DPS increases ERK phosphorylation in hippocampus and prefrontal cortex of mice

Fig. 3 shows the effect of acute treatment with **F-DPS** (50 mg/kg, i.g., 30 min) on ERK1/2 phosphorylation in hippocampus of mice. An unpaired *t*-test revealed a significant difference between Control and **F-DPS** groups for pERK1 [$t_{(6)} = 3.86$, $P < 0.01$] and pERK2 [$t_{(6)} = 7.45$, $P < 0.001$] levels in hippocampus of mice. ERK1 phosphorylation was increased around of 100% whilst pERK2 levels showed an increase of 121%.

In a similar way, pERK1/2 levels were also increased in prefrontal cortex after treatment with **F-DPS** [$t_{(6)} = 5.50$, $P < 0.01$ and $t_{(6)} = 17.96$, $P < 0.001$, respectively; Fig. 4]. A single dose of 50 mg/kg **F-DPS**, given by i.g. route 30 min earlier, was effective in increasing phosphorylated ERK1 (61%) and ERK2 (111%) levels in prefrontal cortex of mice.

The increase on phosphorylation of ERK1/2 after a single dose of **F-DPS** was not accompanied by CREB phosphorylation. Phosphorylated CREB levels were not modified by single **F-DPS** treatment both in hippocampus [$t_{(6)} = 0.53$, $P > 0.05$; Fig. 3] and prefrontal cortex [$t_{(6)} = 2.12$, $P > 0.05$; Fig. 4]. Similarly, there was not any modulation on pCREB expression after repeated treatment with **F-DPS**. An unpaired *t*-test revealed no difference between vehicle and **F-DPS** administration (3 doses of 50 mg/kg) on phosphorylation of CREB in hippocampus [$t_{(6)} = 2.12$, $P > 0.05$] and prefrontal cortex [$t_{(6)} = 1.31$, $P > 0.05$] (Fig. 5).

We performed immunofluorescence experiments to detect the expression and localisation of p-ERK in the hippocampus after acute F-DPS administration. p-ERK immunolabeling was detected in both CA3 (Fig. 6A and C) region and dentate gyrus (Fig. 6E and G). F-DPS selectively increased ERK phosphorylation within the dentate gyrus (Fig. 6F and H) whereas in the CA3 region the number of p-ERK positive cells following F-DPS treatment (Fig. 6B and D) were comparable to that of control (Fig. 6A and C).

3.3. Inhibition of ERK phosphorylation blocks antidepressant-like action of F-DPS in the mouse TST

Fig. 7 shows the effect of acute treatment with **F-DPS** on immobility time and latency for the first episode of immobility in the TST. One-way ANOVA yielded a significant effect of **F-DPS** on total immobility time [$F_{(3,27)} = 21.47$, $P < 0.001$; Fig. 7A] and latency for the first episode of immobility [$F_{(3,7)} = 10.73$, $P < 0.001$; Fig. 7B]. Animals treated with **F-DPS** at doses of 25 and 50 mg/kg, given 30 min earlier by i.g. route, showed a reduction in the total immobility duration around of 52.4%, ($P < 0.001$) and 62.4%, ($P < 0.001$), respectively. Latency in the TST was increased after acute treatment with **F-DPS** at doses of 25 mg/kg (42.5%, $P < 0.01$) and 50 mg/kg (58.4%, $P < 0.001$). A dose of 10 mg/kg was not effective in the TST ($P > 0.05$).

Two-way ANOVA of total immobility time in the TST revealed a significant PD98,059 × **F-DPS** interaction [$F_{(1,20)} = 4.97$, $P < 0.05$]. Results depicted in Fig. 8 show that pretreatment of mice with an inhibitor of MEK, PD98,059 (20 μg/site, i.c.v, 60 min before **F-DPS** treatment), was effective in reversing the anti-immobility effect of **F-DPS** (50 mg/kg, i.g.) in the mouse TST ($P < 0.01$).

4. Discussion

In the present study, we provided further evidence that acute administration of 3-chalcogen selenophenes exerts antidepressant-like effect in mice. **H-DPS**, **CH_3-DPS**, **Cl-DPS**, **F-DPS** and **CF_3-DPS** significantly reduced immobility in the FST without any locomotor-enhancing effect. A close inspection of the results also revealed that the fluorophenyl portion appears to contribute for the potency of selenophene compounds since **F-DPS** was effective at lower doses than other compounds. Furthermore, acute treatment with **F-DPS** promoted a significant increase on phosphorylated ERK levels in prefrontal cortex and hippocampus of mice. Besides, the antidepressant-like effect of **F-DPS** evaluated in the TST was blocked by PD98,059, an inhibitor of ERK phosphorylation (MEK inhibitor). Taken together, the results of this study indicated that ERK phosphorylation is involved in the acute antidepressant-like action of **F-DPS** in mice.

Among all animal models, the FST remains one of the most used tools for screening antidepressants (Petit-Demouliere et al., 2005). Selenophenes tested in this study were effective in reducing total immobility and increasing latency in the FST, suggesting an antidepressant-like action (Costa et al., 2013; Petit-Demouliere et al., 2005). Since locomotor changes may give false positive effects in this test, animals were observed in the locomotor activity monitor, which demonstrated that selenophenes did not change the number of crossings, rearings, velocity and total distance travelled. Thus it is unlikely that the effect of selenophenes observed in the FST is based on stimulation of general motor

Table 2
Locomotor parameters of mice acutely treated with selenophene compounds (dose-range: 10–50 mg/kg).

	Number of crossings	Number of rearings	Velocity (mm/s)	Distance (dm)
H-DPS (mg/kg, i.g., 60 min)				
0	358.7 ± 42.0	10.0 ± 1.8	25.1 ± 3.8	62.2 ± 9.0
10	364.4 ± 47.7	10.4 ± 1.6	29.5 ± 4.2	70.0 ± 9.1
25	319.8 ± 62.4	8.0 ± 1.6	23.9 ± 5.0	55.1 ± 12.2
50	366.3 ± 42.7	12.6 ± 2.5	30.5 ± 1.0	64.2 ± 5.7
CH_3-DPS (mg/kg, i.g., 60 min)				
0	359.7 ± 40.5	9.2 ± 2.5	25.5 ± 5.9	57.1 ± 13.5
10	346.7 ± 37.2	11.8 ± 2.1	27.5 ± 2.4	64.6 ± 5.6
25	327.8 ± 62.4	11.6 ± 2.8	34.1 ± 2.5	70.3 ± 12.7
50	373.7 ± 55.8	10.7 ± 2.7	29.2 ± 2.6	60.8 ± 9.5
Cl-DPS (mg/kg, i.g., 30 min)				
0	404.8 ± 55.9	11.5 ± 2.3	31.6 ± 4.8	73.8 ± 11.9
10	406.5 ± 65.9	12.3 ± 1.4	35.2 ± 4.8	84.2 ± 11.6
25	401.5 ± 36.2	15.5 ± 1.7	36.9 ± 2.7	82.2 ± 10.9
50	406.0 ± 49.0	15.3 ± 1.4	39.2 ± 3.4	90.0 ± 9.8
F-DPS (mg/kg, i.g., 30 min)				
0	349.8 ± 35.1	10.3 ± 1.1	24.9 ± 3.2	53.3 ± 9.0
10	367.7 ± 54.6	10.0 ± 2.3	32.2 ± 4.9	62.8 ± 6.6
25	365.8 ± 49.5	12 ± 2.6	27.5 ± 4.4	70.2 ± 10.9
50	332.7 ± 49.7	12.5 ± 2.0	26.5 ± 3.3	66.9 ± 9.0
CF_3-DPS (mg/kg, i.g., 30 min)				
0	389.7 ± 40.9	11.1 ± 1.6	27.2 ± 4.0	60.9 ± 9.1
10	402.2 ± 42.0	9.0 ± 1.4	24.4 ± 3.7	46.7 ± 7.9
25	394.3 ± 56.6	9.9 ± 1.8	24.3 ± 3.2	53.4 ± 7.9
50	364.8 ± 43.1	10.2 ± 1.2	28.3 ± 3.5	60.8 ± 9.8

Selenophene compounds were administered by intragastric (i.g.) route 30 or 60 min before evaluation in the locomotor activity monitor. Values are expressed as mean ± S.E.M of 6–8 animals/group. Data were analysed by using a one-way analysis of variance (ANOVA), followed by Newman–Keuls test.

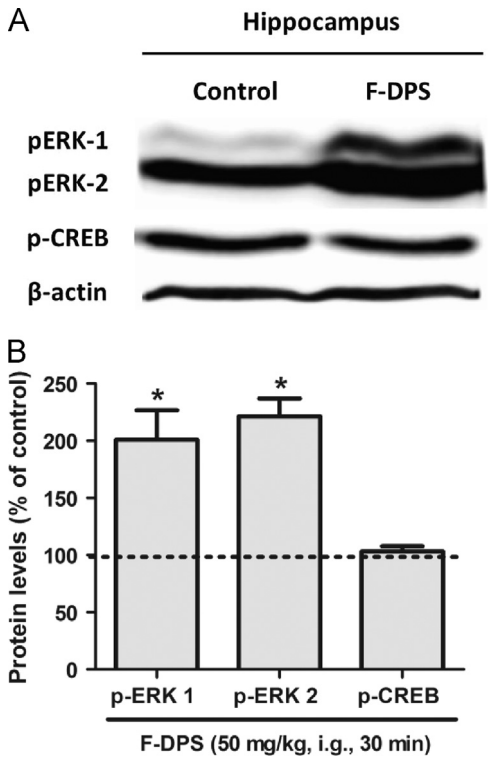

Fig. 3. Effect of acute treatment with **F-DPS** on phosphorylation of ERK1/2 and CREB in hippocampus of mice. **F-DPS** was administered at a dose of 50 mg/kg, i.g and hippocampus was removed after 30 min of treatment. Values are expressed as mean ± S.E.M. of 4 animals. Data were analyzed by using unpaired *t*-test. Asterisk denotes the significance level when compared to the control group: (*) $P < 0.05$. Representative qualitative Western blotting analysis at the top of the figure, graphic shows representative quantification of the proteins immunocontent normalised to β-actin protein.

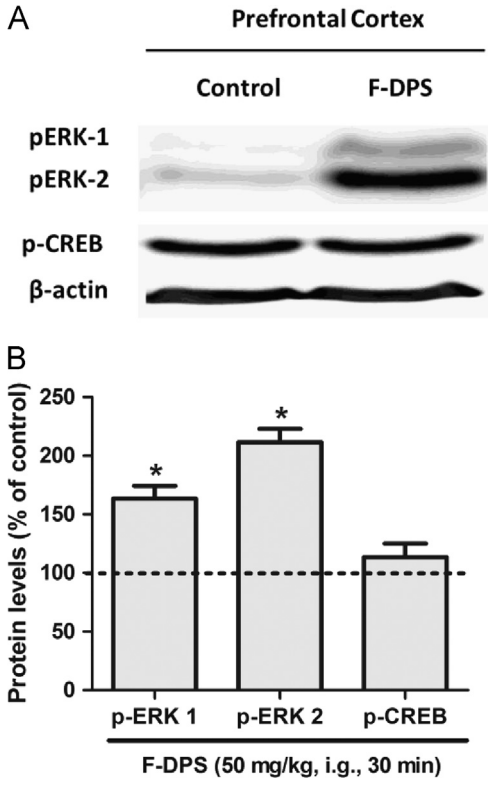

Fig. 4. Effect of acute treatment with **F-DPS** on phosphorylation of ERK1/2 and CREB in prefrontal cortex of mice. **F-DPS** was administered at a dose of 50 mg/kg, i.g and prefrontal cortex was removed after 30 min of treatment. Values are expressed as mean ± S.E.M. of 4 animals. Data were analyzed by using unpaired *t*-test. Asterisk denotes the significance level when compared to the control group: (*) $P < 0.05$. Representative qualitative Western blotting analysis at the top of the figure, graphic shows representative quantification of the proteins immunocontent normalised to β-actin protein.

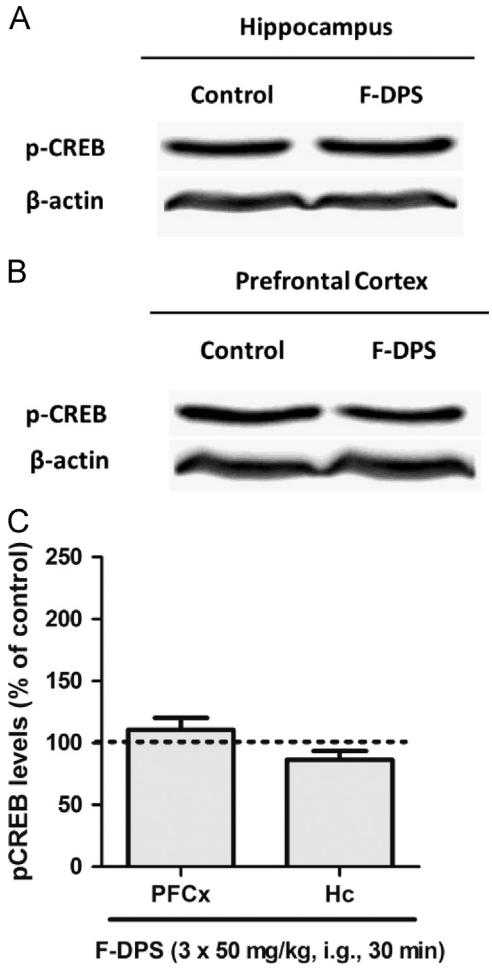

Fig. 5. Effect of repeated treatment with **F-DPS** on CREB phosphorylation in hippocampus and prefrontal cortex of mice. **F-DPS** was administered at the dose of 50 mg/kg, i.g, during 3 days. Hippocampus and prefrontal cortex were removed 30 min after the last dose of **F-DPS**. Values are expressed as mean ± S.E.M. of 4 animals. Data were analyzed by using unpaired *t*-test. Representative qualitative Western blotting analysis at the top of the figure, graphic shows representative quantification of the proteins immunocontent normalised to β-actin protein.

activity, providing evidence that 3-chalcogen selenophenes have antidepressant-like action in mice.

Accordingly, as previously demonstrated by our research group, the administration of selenophenes **Cl-DPS**, **F-DPS** and **CF_3-DPS** (50 mg/kg, i.g. route) reduced significantly the total immobility time of mice when they were administered at 30 min before FST

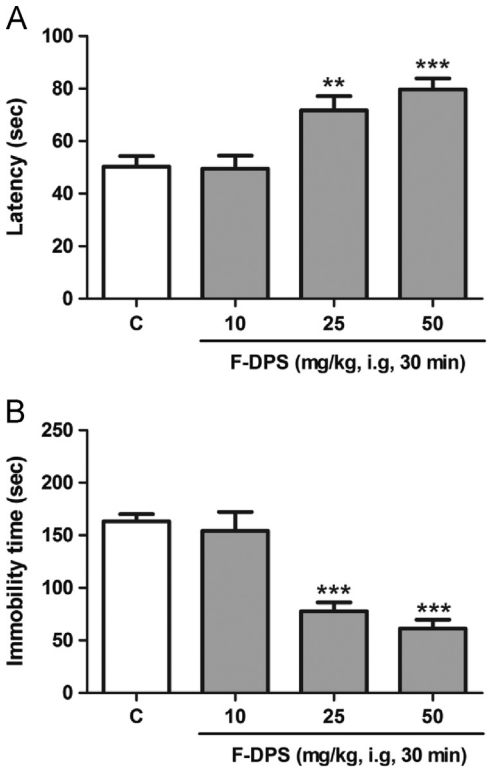

Fig. 7. Effect of acute treatment with **F-DPS** on depression-related behavior in the TST. (A) represents latency to the first episode of immobility and (B) shows the total immobility time. F-DPS (10–50 mg/kg, i.g.) was administered 30 min before testing. Values are expressed as mean ± S.E.M. of 6 animals. Data were analyzed by using a one-way analysis of variance (ANOVA), followed by the Newman–Keuls test. Asterisks denote the significance level when compared to the control group: (**) $P < 0.01$ and (***) $P < 0.001$.

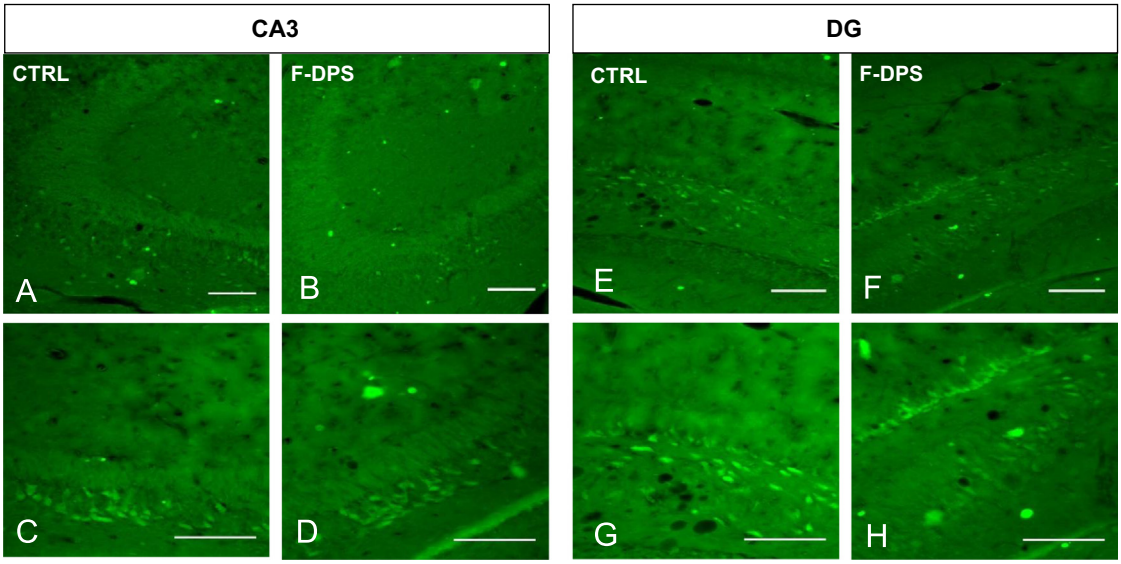

Fig. 6. Phospho-ERK (pERK) labeling in the dorsal hippocampal formation of F-DPS-treated mice. **F-DPS** was administered at the dose of 50 mg/kg, thirty minutes before brain perfusion. In CA3, F-DPS-induced pERK immunostaining (B, low magnification; D, high magnification) is comparable to that of control mice (A, low magnification; C, high magnification). In the dentate gyrus (DG), the number of labeled neurons is substantially increased in the F-DPS-treated mice (F, low magnification; H, high magnification) in comparison with control mice (E, low magnification; G, high magnification). Scale bars 100 μm.

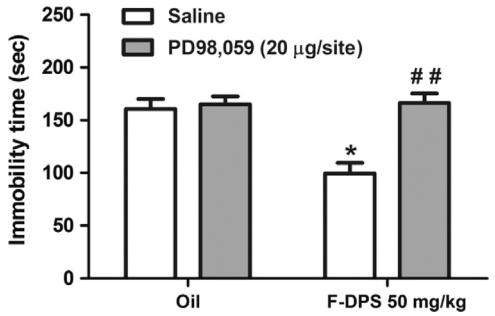

Fig. 8. Effect of ERK phosphorylation inhibition on **F-DPS**-induced reduction in total immobility time in the TST. F-DPS was administered by intragastric (i.g.) route 60 min after PD98,059 (20 μg/site) and 30 min before the test. PD98,059 was injected by intracerebroventricular (i.c.v.) way. Values are expressed as mean ± S.E.M. of 6 animals. Asterisk denotes the significance levels when compared to the control group treated with oil: (*) $P < 0.05$. Hashtags denote the significance levels when compared to the **F-DPS**-treated group: (##) $P < 0.01$ (two-way ANOVA followed by the Newman–Keuls test).

(Gai et al., 2012). Besides confirming these data, in the present study, our results showed that the anti-immobility effect of **Cl-DPS** was maintained up to 180 min after administration, whilst the antidepressant-like action induced by both **F-DPS** and **CF_3-DPS** was significant at only 30 min. Regarding effects of **H-DPS** and **CH_3-DPS**, in the present study we showed for the first time the ability of these selenophenes in reducing the despair behavior in the mouse FST. Recently, we have already demonstrated that administration of **H-DPS** and **CH_3-DPS** at a dose of 50 mg/kg, 30 min before test, was not effective in reducing immobility time (Gai et al., 2012), which was confirmed in the present study. However, when the test was carried out at 60 and 120 min after treatment, both compounds showed a significant antidepressant-like action. These data further appoint to a significant structure–activity relationship and suggest that chemical modifications performed on selenophene structures could alter their pharmacological profile, mainly due pharmacokinetic changes. In order to confirm this hypothesis, our research group is already performing a research project to investigate the absorption, distribution, metabolism and/or excretion profile of selenophene compounds.

Despite the fast and short-acting of **F-DPS**, this compound produced antidepressant-like action at lower doses than **H-DPS**, **CH_3-DPS**, **Cl-DPS** and **CF_3-DPS**. In fact, fluorine has played a particularly important and historical role in the development of biologically active agents and the presence of 4-fluorophenyl group appears to be essential for optimum potency of some neuroleptic agents (Granger and Albu, 2005; Kirk, 2006). Fluorophenyl group is also found in the structure of other antidepressant drugs such as serotonine reuptake inhibitors, like paroxetine, citalopram and escitalopram. Interestingly, recent studies have demonstrated the involvement of serotonergic system in the antidepressant-like action of **F-DPS** (Gai et al., 2013; Gay et al., 2010). Acute anti-immobility effect of this selenophene compound was significantly blocked by serotoninergic receptor antagonists and both acute and subchronic treatments with **F-DPS** inhibited serotonin uptake in a synaptosomal preparation from prefrontal cortex and hippocampus of mice (Gai et al., 2013; Gay et al., 2010). Thus, we considered **F-DPS** as the most promising selenophene to further investigate the mechanisms involved on its antidepressant-like action.

In addition to effects on monoamines, novel theories propose that signal pathway related to synaptic plasticity may be the mechanism of antidepressant action. It is now well established that antidepressants affect different signalling pathways like that producing phosphorylation of CREB (Alboni et al., 2010; Blendy, 2006; Carreno and Frazer, 2014; First et al., 2013; Kuo et al., 2013; Reus et al., 2011). Previous researches indicated that the ERK-CREB signal system may be involved in the molecular mechanism of depression (Qi et al., 2006) and some antidepressant therapies increase both phosphorylated ERK and CREB levels in brain of rodents after repeated treatment (Carreno and Frazer, 2014; Musazzi et al., 2010; Qi et al., 2008; Tardito et al., 2009). Wherever, while early peak of ERK activation is commonly observed, CREB phosphorylation is generally seen only after days or weeks after antidepressant treatment (Di Benedetto et al., 2012). Although **F-DPS** did not modulate pCREB levels, even after repeated administration, pERK levels were significantly increased after acute treatment. ERK is the most-studied member of the MAPK family, and the ERK pathway is the major convergence point in all signal pathways, regulating cellular growth and differentiation and neuronal plasticity. ERK1 and ERK2 are prominently found in hippocampus and prefrontal cortex which are brain regions most likely to be implicated in stress response and depression (Ortiz et al., 1995). It was previously reported that repeated treatment with fluoxetine, a classical antidepressant drug, increases phosphorylation of ERK1/2 but not non-phosphorylated proteins in prefrontal cortex and hippocampus of rats (Qi et al., 2008; Tardito et al., 2009). Interestingly, we observed a significant increase (around 2 times) of both pERK1 and pERK2 isoforms in prefrontal cortex and hippocampus of mice after acute treatment with **F-DPS**. Immunofluorescence experiments further confirmed the ERK1/2 phosphorylation within hippocampus following **F-DPS** treatment and supported the hypothesis of an involvement of hippocampal ERK-mediated pathways in the pharmacological mechanisms of this selenophene. Hippocampal plasticity is integrally involved in the pathophysiology of depression and clinical evidence includes reports of reduced hippocampal volume in magnetic resonance imaging studies and post-mortem studies of depressive patients. Meta-analysis of 32 publications found that volume is significantly reduced with greater than one lifetime major depressive episode or greater than 2 years of illness, suggesting that the observed atrophy is resultant from the burden of illness rather than being a pre-existing risk factor (McKinnon et al., 2009). Specifically, we detected a selective F-DPS-induced ERK overphosphorylation in the dentate gyrus. This appeared of particular importance since the dentate gyrus is the area where the adult hippocampal neurogenesis, a process downregulated by stress conditions and depressive-like behaviours and upregulated by antidepressants, such as SSRI (David et al., 2009; Santarelli et al., 2003), occurred.

Since our results clearly demonstrated that **F-DPS** modulates ERK activation, the next step in this research was to investigate whether this effect could be responsible for its antidepressant-like action. In fact, pretreatment of mice with PD98,059, an inhibitor of ERK phosphorylation, was effective in blocking the acute anti-immobility effect of **F-DPS** in the TST. Based on these data our results suggest that the ERK pathway may be the potential target of this organoselenium compound and participate in the molecular mechanism of its antidepressant-like action.

ERK activity is able to induce phosphorylation of CREB at a specific serine residue, serine 133, producing an active transcription complex enabling target gene activation. The ability to detect phosphorylation of CREB has been an important means to monitor signalling pathways that trigger CREB activation; however, data suggest that CREB-mediated gene expression might occur in the absence of serine 133 phosphorylation (Conkright et al., 2003). Thus, although phosphorylation is an indicator of CREB activation, the ultimate measure of CREB function is gene transcription. As we did not perform mRNA studies, one cannot exclude the modulation in the function of CREB after treatment with **F-DPS**. Nevertheless, it is possible that short-term antidepressant-like action of this organoselenium compound is independent of CREB modulation. Recent studies have demonstrated that some antidepressant drugs increase expression and release of neurotrophic factors via

ERK activity (First et al., 2013; Hisaoka et al., 2007). Besides, serotonin has been showed to increase ERK activation and neurotrophines release via 5-HT_{2A} receptors (Hisaoka et al., 2007). Trophic factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) regulate neurogenesis and neuronal plasticity, which are also improved after antidepressant treatment (Mahar et al., 2014; Ruan et al., 2013). Thus, these data allow us to hypothesise that antidepressant-like action of **F-DPS** might be due to phosphorylation of ERK via activation of serotonergic receptors leading to neurotrophic factors expression/release. In order to confirm it, the effect of selenophene compounds on the hippocampal expression of BDNF and GDNF is target of our research group in future investigations.

5. Conclusion

Our results confirm that the acute antidepressant-like action of 3-chalcogen selenophenes is linked to their chemical structure. **F-DPS** was the most promising selenophene tested and its antidepressant-like action seems to involve ERK signalling activation, particularly in the prefrontal cortex and dentate gyrus of the hippocampus. Our research group is engaged on investigating the pharmacokinetics properties as well as understanding the molecular effects of selenophene compounds that could be further involved on their antidepressant-like action.

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References

Alboni, S., Benatti, C., Capone, G., Corsini, D., Caggia, F., Tascedda, F., Mendlewicz, J., Brunello, N., 2010. Time-dependent effects of escitalopram on brain derived neurotrophic factor (BDNF) and neuroplasticity related targets in the central nervous system of rats. Eur. J. Pharmacol. 643, 180–187.

Blendy, J.A., 2006. The role of CREB in depression and antidepressant treatment. Biol. Psychiatry 59, 1144–1150.

Bruning, C.A., Souza, A.C., Gai, B.M., Zeni, G., Nogueira, C.W., 2011. Antidepressant-like effect of m-trifluoromethyl-diphenyl diselenide in the mouse forced swimming test involves opioid and serotonergic systems. Eur. J. Pharmacol. 658, 145–149.

Carreno, F.R., Frazer, A., 2014. Activation of signalling pathways downstream of the brain-derived neurotrophic factor receptor, TrkB, in the rat brain by vagal nerve stimulation and antidepressant drugs. Int. J. Neuropsychopharmacol. 17, 247–258.

Conkright, M.D., Canettieri, G., Screaton, R., Guzman, E., Miraglia, L., Hogenesch, J.B., Montminy, M., 2003. TORCs: transducers of regulated CREB activity. Mol. Cell 12, 413–423.

Costa, A.P., Vieira, C., Bohner, L.O., Silva, C.F., Santos, E.C., De Lima, T.C., Lino-de-Oliveira, C., 2013. A proposal for refining the forced swim test in Swiss mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 45, 150–155.

David, D.J., Samuels, B.A., Rainer, Q., Wang, J.W., Marsteller, D., Mendez, I., Drew, M., Craig, D.A., Guiard, B.P., Guilloux, J.P., Artymyshyn, R.P., Gardier, A.M., Gerald, C., Antonijevic, I.A., Leonardo, E.D., Hen, R., 2009. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 62, 479–493.

Di Benedetto, B., Kuhn, R., Nothdurfter, C., Rein, T., Wurst, W., Rupprecht, R., 2012. N-desalkylquetiapine activates ERK1/2 to induce GDNF release in C6 glioma cells: a putative cellular mechanism for quetiapine as antidepressant. Neuropharmacology 62, 209–216.

Dias, G.R., de Almeida, T.M., Sudati, J.H., Dobrachinski, F., Pavin, S., Soares, F.A., Nogueira, C.W., Barbosa, N.B., 2014. Diphenyl diselenide supplemented diet reduces depressive-like behavior in hypothyroid female rats. Physiol. Behav. 124, 116–122.

Dwivedi, Y., Rizavi, H.S., Roberts, R.C., Conley, R.C., Tamminga, C.A., Pandey, G.N., 2001. Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. J. Neurochem. 77, 916–928.

Feng, P., Guan, Z., Yang, X., Fang, J., 2003. Impairments of ERK signal transduction in the brain in a rat model of depression induced by neonatal exposure of clomipramine. Brain Res. 991, 195–205.

First, M., Gil-Ad, I., Taler, M., Tarasenko, I., Novak, N., Weizman, A., 2013. The effects of reboxetine treatment on depression-like behavior, brain neurotrophins, and ERK expression in rats exposed to chronic mild stress. J. Mol. Neurosci. 50, 88–97.

Gai, B.M., Bortolatto, C.F., Bruning, C.A., Zborowski, V.A., Stein, A.L., Zeni, G., Nogueira, C.W., 2014. Depression-related behavior and mechanical allodynia are blocked by 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene in a mouse model of neuropathic pain induced by partial sciatic nerve ligation. Neuropharmacology 79, 580–589.

Gai, B.M., Bortolatto, C.F., Heck, S.O., Stein, A.L., Duarte, M.M., Zeni, G., Nogueira, C.W., 2013. An organoselenium compound improves behavioral, endocrinal and neurochemical changes induced by corticosterone in mice. Psychopharmacology 231, 2119–2130.

Gai, B.M., Stein, A.L., Roehrs, J.A., Bilheri, F.N., Nogueira, C.W., Zeni, G., 2012. Synthesis and antidepressant-like activity of selenophenes obtained via iron (III)–PhSeSePh-mediated cyclization of Z-selenoenynes. Org. Biomol. Chem 10, 798–807.

Gay, B.M., Prigol, M., Stein, A.L., Nogueira, C.W., 2010. Antidepressant-like pharmacological profile of 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene: involvement of serotonergic system. Neuropharmacology 59, 172–179.

Gornall, A.G., Bardawill, C.J., David, M.M., 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 177, 751–766.

Granger, B., Albu, S., 2005. The haloperidol story. Ann. Clin. Psychiatry 17, 137–140.

Haley, T.J., McCormick, W.G., 1957. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br. J. Pharmacol. Chemother. 12, 12–15.

Hisaoka, K., Takebayashi, M., Tsuchioka, M., Maeda, N., Nakata, Y., Yamawaki, S., 2007. Antidepressants increase glial cell line-derived neurotrophic factor production through monoamine-independent activation of protein tyrosine kinase and extracellular signal-regulated kinase in glial cells. J. Pharmacol. Exp. Ther. 321, 148–157.

Kirk, K.L., 2006. Selective fluorination in drug design and development: an overview of biochemical rationales. Curr. Top. Med. Chem. 6, 1447–1456.

Kuo, J.R., Cheng, Y.H., Chen, Y.S., Chio, C.C., Gean, P.W., 2013. Involvement of extracellular signal regulated kinases in traumatic brain injury-induced depression in rodents. J. Neurotrauma 30, 1223–1231.

Laursen, S.E., Belknap, J.K., 1986. Intracerebroventricular injections in mice. Some methodological refinements. J. Pharmacol. Methods 16, 355–357.

Mahadevan, J., Parazzoli, S., Oseid, E., Hertzel, A.V., Bernlohr, D.A., Vallerie, S.N., Liu, C.Q., Lopez, M., Harmon, J.S., Robertson, R.P., 2013. Ebselen treatment prevents islet apoptosis, maintains intranuclear Pdx-1 and MafA levels, and preserves beta-cell mass and function in ZDF rats. Diabetes 62, 3582–3588.

Mahar, I., Bambico, F.R., Mechawar, N., Nobrega, J.N., 2014. Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. Neurosci. Biobehav. Rev. 38, 173–192.

McKinnon, M.C., Yucel, K., Nazarov, A., MacQueen, G.M., 2009. A meta-analysis examining clinical predictors of hippocampal volume in patients with major depressive disorder. J. Psychiatry Neurosci. 34, 41–54.

Mebratu, Y., Tesfaigzi, Y., 2009. How ERK1/2 activation controls cell proliferation and cell death: is subcellular localization the answer? Cell Cycle 8, 1168–1175.

Millan, M.J., 2004. The role of monoamines in the actions of established and "novel" antidepressant agents: a critical review. Eur. J. Pharmacol. 500, 371–384.

Musazzi, L., Mallei, A., Tardito, D., Gruber, S.H., El Khoury, A., Racagni, G., Mathe, A.A., Popoli, M., 2010. Early-life stress and antidepressant treatment involve synaptic signaling and Erk kinases in a gene-environment model of depression. J. Psychiatr. Res. 44, 511–520.

Nogueira, C.W., Rocha, J.B., 2011. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. Arch. Toxicol. 85, 1313–1359.

Ortiz, J., Harris, H.W., Guitart, X., Terwilliger, R.Z., Haycock, J.W., Nestler, E.J., 1995. Extracellular signal-regulated protein kinases (ERKs) and ERK kinase (MEK) in brain: regional distribution and regulation by chronic morphine. J. Neurosci. 15, 1285–1297.

Petit-Demouliere, B., Chenu, F., Bourin, M., 2005. Forced swimming test in mice: a review of antidepressant activity. Psychopharmacology 177, 245–255.

Pinton, S., Sampaio, T.B., Ramalho, R.M., Rodrigues, C.M., Nogueira, C.W., 2013. p,p′-Methoxyl-diphenyl diselenide prevents neurodegeneration and glial cell activation induced by streptozotocin in rats. J. Alzheimer's Dis. 33, 133–144.

Porsolt, R.D., Bertin, A., Blavet, N., Deniel, M., Jalfre, M., 1979. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. Eur. J. Pharmacol. 57, 201–210.

Qi, X., Lin, W., Li, J., Li, H., Wang, W., Wang, D., Sun, M., 2008. Fluoxetine increases the activity of the ERK-CREB signal system and alleviates the depressive-like behavior in rats exposed to chronic forced swim stress. Neurobiol. Dis. 31, 278–285.

Qi, X., Lin, W., Li, J., Pan, Y., Wang, W., 2006. The depressive-like behaviors are correlated with decreased phosphorylation of mitogen-activated protein kinases in rat brain following chronic forced swim stress. Behav. Brain Res. 175, 233–240.

Reus, G.Z., Stringari, R.B., Ribeiro, K.F., Ferraro, A.K., Vitto, M.F., Cesconetto, P., Souza, C.T., Quevedo, J., 2011. Ketamine plus imipramine treatment induces antidepressant-like behavior and increases CREB and BDNF protein levels and PKA and PKC phosphorylation in rat brain. Behav. Brain Res. 221, 166–171.

Roux, P.P., Blenis, J., 2004. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol. Mol. Biol. Rev. 68, 320–344.

Ruan, L., Lau, B.W., Wang, J., Huang, L., Zhuge, Q., Wang, B., Jin, K., So, K.F., 2013. Neurogenesis in neurological and psychiatric diseases and brain injury: from bench to bedside. Prog. Neurobiol..

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C., Hen, R., 2003. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 301, 805–809.

Stein, A.L., Alves, D., da Rocha, J.T., Nogueira, C.W., Zeni, G., 2008. Copper iodide-catalyzed cyclization of (Z)-chalcogenoenynes. Org. Lett. 10, 4983–4986.

Steru, L., Chermat, R., Thierry, B., Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85, 367–370.

Tardito, D., Musazzi, L., Tiraboschi, E., Mallei, A., Racagni, G., Popoli, M., 2009. Early induction of CREB activation and CREB-regulating signalling by antidepressants. Int. J. Neuropsychopharmacol. 12, 1367–1381.

Wilhelm, E.A., Souza, A.C., Gai, B.M., Chagas, P.M., Roehrs, J.A., Nogueira, C.W., 2012. Hyperthermic seizures enhance responsiveness to pentylenetetrazole and induce cognitive dysfunction: protective effect of 3-alkynyl selenophene. Life Sci. 90, 666–672.