Revista Brasileira de Farmacognosia 25 (2015) 387–394

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.sbfgnosia.org.br/revista

Original Article

G-protein activation revealed by [^{35}S]-GTPγS binding assay is involved on the antidepressant-like effect of *Hypericum caprifoliatum* and *Hypericum polyanthemum* cyclohexane extracts

Alice Fialho Viana[a], Jean Costentin[b], Jean-Claude do Rego[b,c], Stela Maris Kuze Rates[a,*]

[a] *Programa de Pós-graduação em Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil*
[b] *Service Commun d'Analyse Comportementale, Institut de Recherche et d'Innovation Biomédicale IRIB, Faculté de Médecine et Pharmacie, Université de Rouen, Rouen Cedex, France*
[c] *Centre National de la Recherche Scientifique, France*

A R T I C L E I N F O

Article history:
Received 30 May 2015
Accepted 24 June 2015
Available online 26 July 2015

Keywords:
Hypericum
[^{35}S]-GTPγS binding assay
Uliginosin B
Antidepressant
Forced swimming test
Monoamine receptors

A B S T R A C T

Previous studies by us demonstrated the antidepressant-like and antinociceptive effects of lipophilic extracts and dimeric acyl-phloroglucinols from species of the genus *Hypericum* native to Southern Brazil. Uliginosin B and HC1 (an enriched phloroglucinol fraction from *Hypericum caprifoliatum*) are able to inhibit monoamine synaptosomal uptake without binding to the monoaminergic sites on neuronal transporters, unlike classical antidepressants. The current study aimed at investigating the action of *H. caprifoliatum* Cham. & Schltdl. and *Hypericum polyanthemum* Klotzsch ex Reichardt, Hypericaceae, cyclohexane extracts and their main component, HC1 and uliginosin B, on G protein coupled receptors by using the [^{35}S]-guanosine-5′-*O*-(3-thio)triphosphate ([^{35}S]-GTPγS) binding assay, which reveals the G protein activity. The antidepressant-like effect of acute (one or three treatments within 24 h) and repeated (five days with and without a three day wash-out) treatments with the cyclohexane extracts was evaluated using the rat forced swimming test. The [^{35}S]-GTPγS binding to monoamines and opioid receptors stimulated by agonists was performed *ex vivo* in brain membranes of rats acutely or repeatedly treated with the cyclohexane extracts. The effect of HC1 and Uliginosin B on [^{35}S]-GTPγS binding assay was performed by direct incubation with brain membranes in the absence of agonists. Their antidepressant-like effect was evaluated through the mice forced swimming test. The extracts, HC1 and Uliginosin B showed antidepressant-like effect in the forced swimming test. The acute treatments with extracts increased the [^{35}S]-GTPγS binding stimulated by the monoamines, while after five days of treatment the [^{35}S]-GTPγS binding was reduced even after three day wash-out. These effects are not due to HC1 or Uliginosin B interaction with the receptors, since direct incubation with these phloroglucinols did not affect [^{35}S]-GTPγS binding to membranes. Our findings indicate that *H. caprifoliatum* and *H. polyanthemum* extracts bring about adaptive changes in monoamine receptors, which reinforces their antidepressant-like profile.

© 2015 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.

Introduction

Natural products can be a source of substances with innovative mechanisms of action (Rates, 2001; Rollinger et al., 2006). In previous works we demonstrated that *Hypericum caprifoliatum* Cham. & Schltdl. (HCP) and *H. polyanthemum* Klotzsch ex Reichardt (POL), Hypericaceae, cyclohexane extracts have potential antidepressant and antinociceptive effects (Viana et al., 2003, 2005; Stein et al., 2012; Stolz et al., 2012, 2014a,b). *H. caprifoliatum* extracts and its enriched phloroglucinol fraction (HC1) act on pre-synapse by inhibiting monoamine uptake but differently from antidepressants they do not bind to the monoamine sites on neuronal transporters (Viana et al., 2005). Furthermore, the antidepressant like-effect in the forced swimming test (FST) was inhibited by dopamine D_1 and D_2 receptor antagonists (Viana et al., 2005). The antinociceptive effect was blocked by naloxone (opioid receptor antagonist) (Viana et al., 2003). POL and uliginosin B had similar effects, uliginosin B was able to inhibit synaptosomal monoamine uptake without binding to their sites on neuronal transporters (Stein et al., 2012). The pretreatment with dopamine receptor antagonists, α-adrenoceptor antagonists, pCPA (inhibitor of serotonin synthesis) and MK-801 (*N*-methyl-D-aspartic acid receptor antagonist) significantly prevented the HP4 antidepressant and antinociceptive effects (Stein et al., 2012; Stolz et al., 2014a). Recently, Stolz et al.

* Corresponding author.
E-mail: stela.rates@ufrgs.br (S.M.K. Rates).

http://dx.doi.org/10.1016/j.bjp.2015.06.008
0102-695X/© 2015 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. All rights reserved.

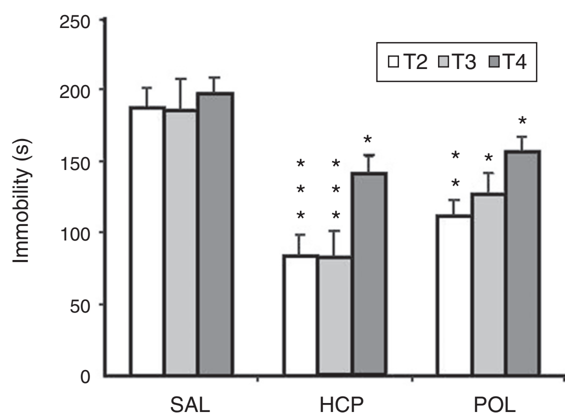

Fig. 1. Effect of T2 (three treatments during 24 h), T3 (two treatments per day during five days) and T4 (two treatments per day during five days followed by a three days wash-out) with 90 mg/kg of *H. caprifoliatum* (HCP), *H. polyanthemum* (POL) or saline (SAL 1 ml/kg) on rat forced swimming test. Data are presented in mean ± SEM ($n = 8$ rats/group). Significantly different values were detected by one way ANOVA followed by post hoc Student–Newman–Keuls test: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ as compared to their respective saline (SAL) group.

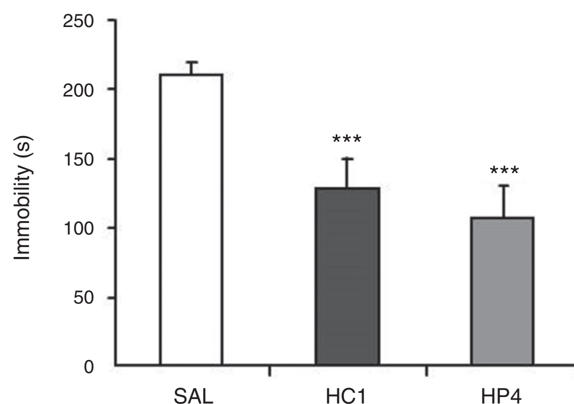

Fig. 2. Effect of acute treatment with the phloroglucinols HC1 (*Hypericum caprifoliatum*) and HP4 (*H. polyanthemum*) 90 mg/kg *p.o.*; or saline (SAL 10 ml/kg) on mice forced swimming test. Data are presented in mean ± SEM ($n = 10$ mice/group). Significantly different values were detected by one way ANOVA followed by post hoc Student–Newman–Keuls test: $^{***}p < 0.001$ as compared to their respective saline (SAL) group.

(2014b) verified that uliginosin B has synergistic effect with morphine, while with amitriptyline or clonidine the interaction is additive. Altogether, these data indicate that the antidepressant and antinociceptive effects of the phloroglucinols from species of *Hypericum* native to Southern Brazil involve the activation of several neurotransmission pathways, but direct evidence of their ability to activate G protein coupled receptors (GPCR) are still lacking.

The knowledge that antidepressant agents affect GPCR function (Donati and Rasenick, 2003; Avissar and Schereiber, 2006; Czysz et al., 2015) prompted us to investigate the effect of *H. caprifoliatum* (HCP) and *H. polyanthemum* (POL) cyclohexane extracts on some GPCR implicated with the mode of action of antidepressant and analgesic drugs by using the [^{35}S]-guanosine-5′-*O*-(3-thio)triphosphate ([^{35}S]-GTPγS) binding assay, which reveals the G protein activity. We tested the effect of acute and repeated administration of HCP (*H. caprifoliatum*) and POL (*H. polyanthemum*) cyclohexane extracts on immobility time in FST and on monoamine and opioid receptor-stimulated [^{35}S]-GTPγS binding in rat brain membranes. We have also investigated the effect the phloroglucinols derivatives, HC1 and uliginosin B on mice FST and carried out the [^{35}S]-GTPγS binding assay after direct incubation of these compounds with brain membranes.

Materials and methods

Plant material and preparation of the extract

The aerial parts of *Hypericum caprifoliatum* Cham. and Schltdl., Hypericaceae, were collected in the region of Viamão (29°57′ S; 50°59′ W), and the ones from *H. polyanthemum* Klotzsch ex Reichardt, Hypericaceae, were collected in the region of Caçapava do Sul (30°54′ S; 53°87′ W). The voucher specimens are deposited in the herbarium of the Federal University of Rio Grande do Sul (ICN) (Bordignon, 1400 and 1429, respectively). Plant collection was authorized by Conselho de Gestão do Patrimônio Genético – CGEN and Instituto Brasileiro do Meio Ambiente – IBAMA – 003/2008 P 02000.001717/2008.60.

Hypericum extracts were prepared as described by Viana et al. (2005) and Stein et al. (2012). The phloroglucinol derivatives, HC1 from *H. caprifoliatum* and uliginosin B (herein named HP4) from *H. polyanthemum* were isolated by means of thin layer and column chromatography on preparative silica gel GF254 (Merck) using chloroform/hexane (3.5:1, v/v) as eluent (Nör et al., 2004; Stolz et al., 2012).

Animals

Adult Male Sprague Dawley rats, weighing (180–200 g), were purchased from IFFA-CREDO/Charles River Laboratories (Domaine des Oncins, Saint-Germain sur L'Arbresle, France). Adult Male CF1 mice (25–35 g) from Fundação Estadual de Produção e Pesquisa em Saúde (FEPPS-RS, Brazil) breeding colony were used. The rats were housed by five in Makrolon cages (42 cm × 27.5 cm × 18 cm); mice were housed in plastic cages (17 cm × 28 cm × 13 cm) in groups of eight mice. All animals were kept under a 12 h light/dark cycle (lights on between 7 a.m. and 7 p.m.) in a well ventilated room, at constant temperature of 22° ± 1 °C, with free access to standard certified rodent diet and tap water. All the behavioral experiments were performed according to guidelines of The National Research Ethical Committee (published by National Heath Council – MS, 1998), which are in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). The behavioral experiments were approved by National Commission of Research Ethics – Brazil (project approval number 01-188 at 07/26/2001).

[^{35}S]-GTPγS binding assay

Rats were submitted to four different treatment regimens with POL or HCP 90 mg/kg, *p.o.*: T1 – one treatment by oral gavage 1 h before the assays; T2 – three treatments within 24 h (23, 5 and 1 h before the sacrifice); T3 – two treatments per day during five days; T4 – two treatments per day during five days followed by a three day wash-out before the assays. The extracts were dissolved in water with 5% polissorbate 80 to a concentration of 90 mg/ml, the volume of administration was 1 ml/kg.

One hour after the last administration (T1, T2 and T3) or three days later (T4) the animals were killed by decapitation. Frontal cortex, striata, hypothalamus and thalamus were removed and homogenized in 20 vol of 0.32 M sucrose. The same brain structures were removed from non-treated rats to be used as control and for direct incubation with HC1 or HP4 (10^{-10}–10^{-6} M). Crude membrane preparations were isolated according to the modified method described elsewhere (Viana et al., 2005). Briefly, the homogenates were centrifuged (1000 × *g* for 15 min); the supernatants from

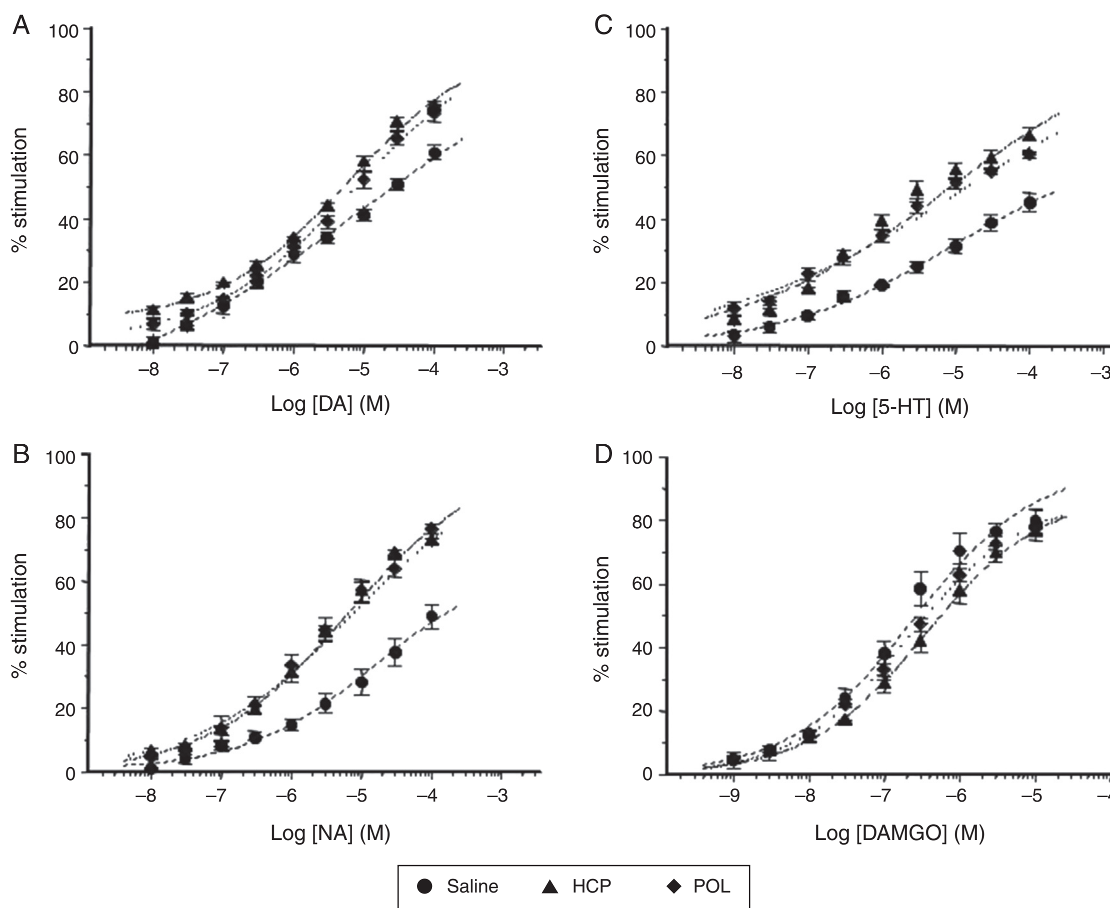

Fig. 3. Effect of T1 (single administration) with HCP, POL (90 mg/kg, *p.o.*) or saline on agonist-stimulated [^{35}S]-GTPγS binding measured *ex vivo*. Membranes prepared from rat striatum (A), hypothalamus (B), frontal cortex (C) and thalamus (D) were incubated with increasing doses of dopamine (A), noradrenaline (B), serotonin (C) or DAMGO (D) in the presence of 0.1 nM [^{35}S]-GTPγS and 10 μM GDP. Data are presented as percentage of binding stimulation over GDP basal stimulation (absence of agonist). Mean ± SEM from four separated experiments carried out in duplicate.

two centrifugations were combined and centrifuged (17,000 × *g* for 30 min). The resulting pellet was then suspended in Tris buffer (50 mM Tris/HCl, 100 mM NaCl, 5 mM $MgCl_2$, and 1 mM EDTA, pH 7.4), sonicated, and centrifuged (50,000 × *g* for 10 min). The final protein concentration was measured by the method of Lowry et al. (1951).

[^{35}S]-GTPγS binding assays were performed as follows. Membranes (15–20 μg/100 μl) were incubated in the assay buffer (Tris buffer added with 1 mM dithiothreitol (DTT) and 0.1% metabisulphite) with 10 μM GDP (Sigma–Aldrich Co.), 10^{-4}–10^{-8} M of dopamine, noradrenaline, serotonin or DAMGO ([D-Ala^2, N-Me-Phe^4, Gly^5-ol]-Enkephalin) in the presence of 0.1 nM [^{35}S]-GTPγS (1250 Ci/mmol; Perkin-Elmer, Courtaboeuf, France) in a total volume of 1 ml. Basal binding was assessed in the absence of monoamines or DAMGO and presence of GDP (guanosine-5′-*O*-(3-thio)diphosphate), and the non-specific binding was assessed in the presence of 10 μM GTPγS (Sigma–Aldrich). In parallel, incubations with HC1 or HP4 in the absence of monoamines or DAMGO were made. The entire mixture was incubated at 25 °C for 2 h and filtered through Whatman GF/B glass fiber filters, which had been pre-soaked for 2 h in Tris buffer, and washed three times with 4 ml of ice-cold Tris buffer, using a Millipore Sampling Manifold (Billerica, MA, USA). Bound radioactivity was determined in Tri-Carb 2100 TR liquid scintillation counter (Packard) after overnight extraction of the filters in 4 ml of Ultima Gold scintillation fluid (Perkin-Elmer). Four independent experiments for each assay were carried out in duplicate.

Forced swimming test

Another batch of rats were submitted to treatments T2, T3 and T4 and evaluated in the FST. In brief, before the beginning of the treatment, rats were submitted to swimming for 15 min in 30 cm deep water with temperature between 23 ± 2 °C. The ambient temperature was approximately 22 °C. At the end of the swimming exposition, the animals were removed from the water and gently dried. One hour after the last treatment (T2 and T3) or three days after treatment discontinuation (T4), the animals were submitted to a second swimming exposure (5 min), and their immobility time was measured. In order to evaluate the phloroglucinols derivatives, HC1 and HP4, antidepressant-like effect, we tested them at the single dose of 90 mg/kg (*p.o.*) on mice FST according to Porsolt et al. (1977, 1978) and Centurião et al. (2014).

Statistical analysis

The data were evaluated using one or two-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test or Student's *t*-test depending on the experimental design. *p*-Values less than 0.05 were considered as statistically significant.

The percent stimulation of [^{35}S]-GTPγS binding was calculated according to the following formula: $(S - B)/B \times 100\%$, where S is the stimulated level and B is the basal level of [^{35}S]-GTPγS binding in the presence of GDP. Individual concentration–response curves were obtained by a non-linear regression analysis.

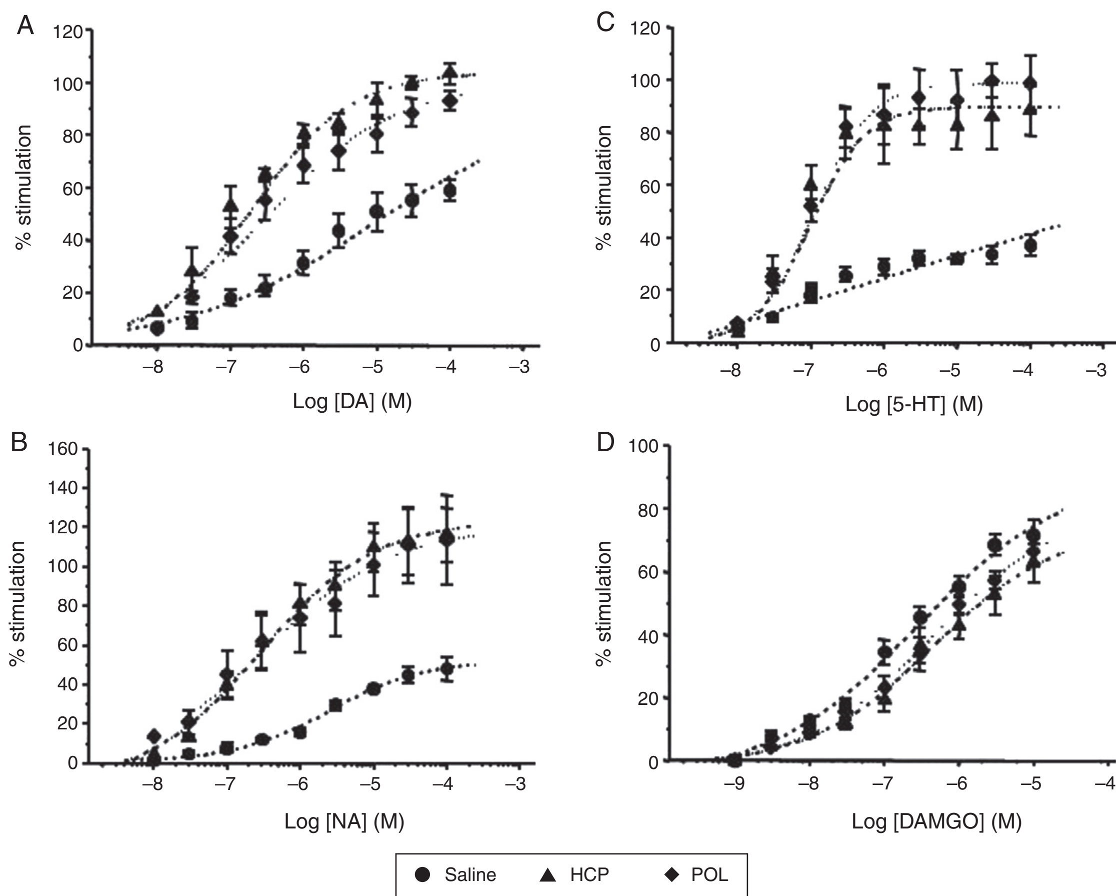

Fig. 4. Effect of T2 (three treatments during 24 h (23, 5 and 1 h before assay) with HCP, POL (90 mg/kg, *p.o.*) or saline on agonist-stimulated [^{35}S]-GTPγS binding measured *ex vivo*. Membranes prepared from rat striatum (A), hypothalamus (B), frontal cortex (C) and thalamus (D) were incubated with increasing doses of dopamine (A), noradrenaline (B), serotonin (C) or DAMGO (D) in the presence of 0.1 nM [^{35}S]-GTPγS and 10 μM GDP. Data are presented as percentage of binding stimulation over GDP basal stimulation (absence of agonist). Mean ± SEM from four separated experiments carried out in duplicate.

Results

Forced swimming test

All treatment regimens with HCP [one-way ANOVA: $F(5,53) = 17.46$; $p < 0.05$] and POL [one-way ANOVA: $F(5,54) = 4.89$; $p < 0.05$] significantly reduced immobility time. The five day treatments (twice a day) were not more effective than the classical Porsolt protocol (T2). The antiimmobility effect remained even after three days wash-out (T4) although less pronounced than those observed in T2 and T5 treatment twice a day (T3) (Fig. 1). Even though there are no statistically significant differences between POL and HCP treatments, it can be observed that the POL effects are weaker than those of HCP (Fig. 1). Both HC1 and HP4 were effective in reducing mice immobility time [one-way ANOVA: $F(2,27) = 12.36$; $p < 0.001$] (Figs. 1 and 2).

[^{35}S]-GTPγS binding assay

Ex vivo experiments

The different treatment regimens induced contrasting results. Treatments T1 and T2 with HCP and POL increased the maximal effect (E_{max}) of [^{35}S]-GTPγS binding induced by all monoamines (Figs. 3 and 4) and did not affect DAMGO curve features. The monoamine potency (EC_{50}) to induce [^{35}S]-GTPγS binding was also increased.

Repeated treatment (T3 and T4) with HCP or POL induced a reduction in the E_{max} and EC_{50} of [^{35}S]-GTPγS binding stimulated by monoamines (Figs. 5 and 6). This reduction was significant for the three monoamines when membranes were prepared one hour after the last treatment (T3) and even following a three day wash-out (T4). DAMGO-stimulated curves were not affected by the treatments.

In vitro *experiments*

In order to verify whether the effects observed *ex vivo* were related to direct phloroglucinols action on receptor functionality, we evaluated the [^{35}S]-GTPγS binding stimulated by HC1 and HP4 in the absence of agonists. HC1 and HP4 were not able to stimulate the binding of [^{35}S]-GTPγS to any of the Gα coupled to receptors in membranes prepared from non-treated animals (data not shown) (Fig. 7).

Discussion

Previous studies by us demonstrated the antidepressant-like and antinociceptive effects of lipophilic extracts and dimeric acyl-phloroglucinols from species of the genus *Hypericum* native to Southern Brazil. The phloroglucinol derivatives, uliginosin B (HP4) and HC1, inhibited monoamine synaptosomal uptake in a concentration-dependent manner, without binding to monoaminergic sites on neuronal transporters, unlike classical antidepressants (Viana et al., 2005; Stein et al., 2012). The fact that *Hypericum* phloroglucinol derivatives from Brazilian and European species (Viana et al., 2005; Chatterjee et al., 1998; Müller et al., 2001) inhibit monoamine uptake by a mechanism different from

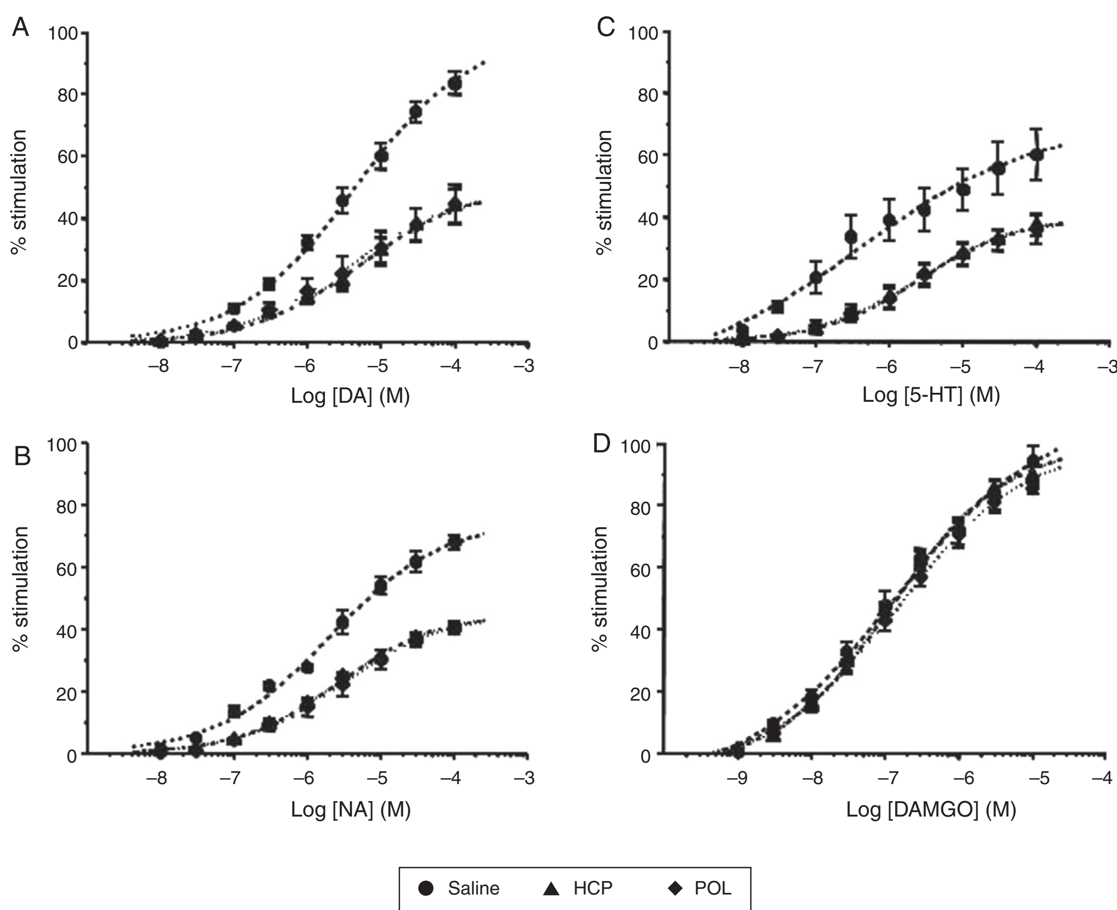

Fig. 5. Effect of T3 (two treatments per day during five days) with HCP, POL (90 mg/kg, *p.o.*) or saline on agonist-stimulated [^{35}S]-GTPγS binding measured *ex vivo*. Membranes prepared from rat striatum (A), hypothalamus (B), frontal cortex (C) and thalamus (D) were incubated with increasing doses of dopamine (A), noradrenaline (B), serotonin (C) or DAMGO (D) in the presence of 0.1 nM [^{35}S]-GTPγS and 10 μM GDP. Data are presented as percentage of binding stimulation over GDP basal stimulation (absence of agonist). Mean ± SEM from four separated experiments carried out in duplicate.

classical antidepressants is coherent with their chemical structure; these molecules do not have nitrogen atom in their structure, which is a core feature for binding to neuronal monoamines transporters (Appell et al., 2004). However, until this moment the molecular mechanisms by which extracts of *Hypericum* species exert their antidepressant-like effects remains unclear.

It is a consensus among the field of psychopharmacology that the increase of monoamine levels in the synaptic clef caused by uptake inhibition is only the first from a series of neurochemical modifications induced by antidepressant. This increase evidently will lead to post synaptic modifications at different levels of the signaling cascades (Schreiber and Avissar, 2003; Millan, 2006; Stahl, 2008). Since antidepressant agents affect GPCRs function (Donati and Rasenick, 2003; Avissar and Schereiber, 2006; Czysz et al., 2015) we decided to investigate the effect of *H. caprifoliatum* (HCP) and *H. polyanthemum* (POL) cyclohexane extracts on monoaminergic receptors response in rat brain by measuring agonist-induced [^{35}S]-GTPγS binding.

Acute and chronic treatments with the extracts showed opposite results regarding agonist-induced [^{35}S]-GTPγS binding: while acute treatments (T1 and T2) increased it, repeated treatments (T3 and T4) diminished it. The [^{35}S]-GTPγS binding increase indicates protein G activation and could be related to receptors response to the sudden increase of monoamine at the synaptic cleft (Lohse et al., 2008). The [^{35}S]-GTPγS binding reduction after 5 days of treatment (T3 and T4) may reveal a regulatory mechanisms resulting from the persistently high monoamines levels at the synaptic cleft (Hermans, 2003; Stahl, 2008), which could also explain the persistent antiimmobility observed in the FST after 3 days of washout. These results are in agreement with many experimental results that show a reduction in [^{35}S]-GTPγS–G-protein coupling after chronic treatment with several classes of antidepressant drugs and monoamine transporter blockers (Hensler, 2002; O'Connor et al., 2005; Castro et al., 2008; Rossi et al., 2008; Vidal et al., 2010).

Receptors, transporters and G-proteins are not quiescent plasma membrane proteins, many evidence support that they dynamically traffic to and from plasmatic membrane, both constitutively as well as in response to psychostimulant exposure, receptor activation and neuronal activity (Melikian, 2004; Chisari et al., 2007; Evans et al., 2010). The GPCR decreased responsiveness after repeated agonist treatment can occur through a variety of mechanisms, including desensitization, down-regulation and redistribution of cell surface receptors (trafficking) (Ferguson, 2001; Harrison and Traynor, 2003; Hermans, 2003; O'Connor et al., 2005). The exact mechanisms that causes neuronal changes following antidepressant treatments are yet unknown. Many studies show that antidepressants may modulate the density of monoaminergic neurotransmitter receptors in brains (Gould and Manji, 2002; Avissar and Schereiber, 2006). It appears that GTP-binding properties of G proteins are unaffected by antidepressant treatment, and their effects on G-protein signaling do not include changes in receptor or G protein gene expression (down regulation). Several studies have demonstrated that the observed desensitization of monoaminergic receptors following chronic antidepressant use is rather due to a decrease in the efficacy of coupling between receptor and

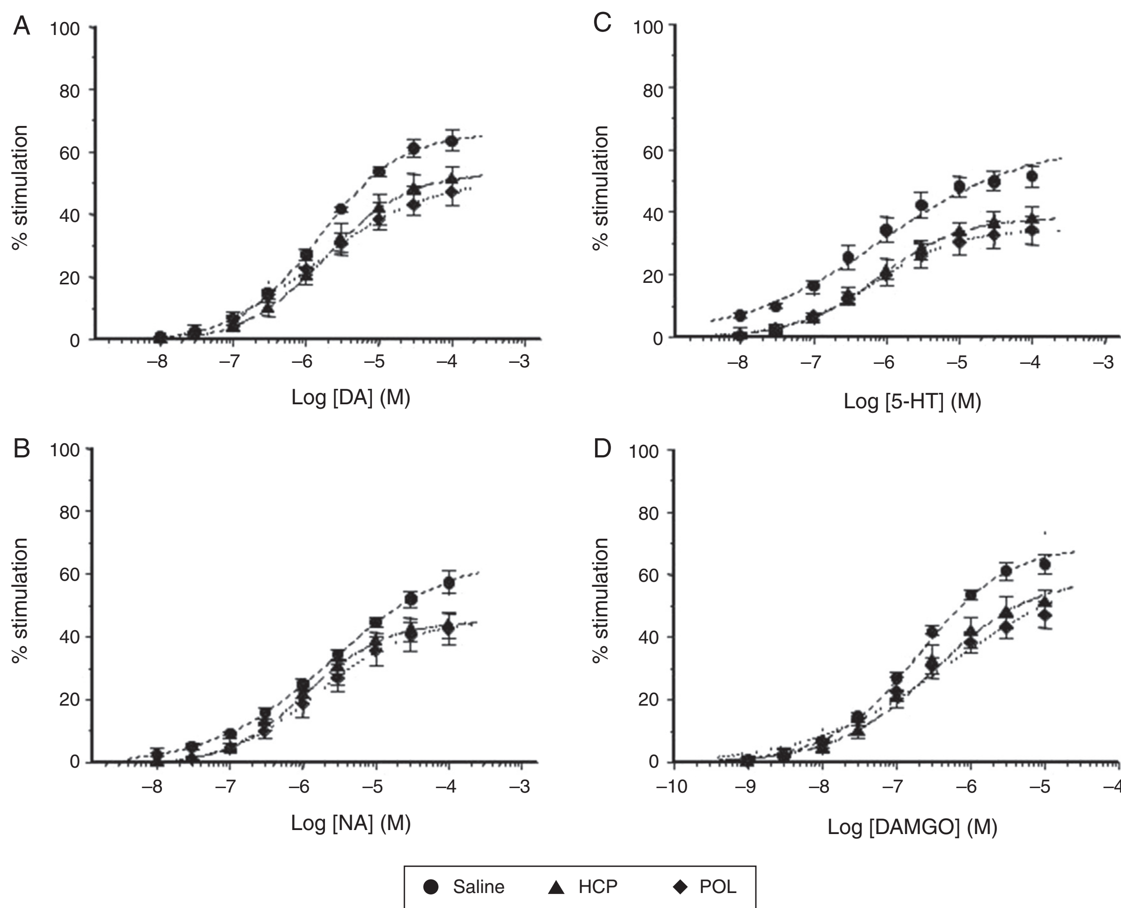

Fig. 6. Effect of T4 (two treatments per day during five days followed by a three days wash-out) with HCP, POL (90 mg/kg, *p.o.*) or saline on agonist-stimulated [^{35}S]-GTPγS binding measured *ex vivo*. Membranes prepared from rat striatum (A), hypothalamus (B), frontal cortex (C) and thalamus (D) were incubated with increasing doses of dopamine (A), noradrenaline (B), serotonin (C) or DAMGO (D) in the presence of 0.1 nM [^{35}S]-GTPγS and 10 μM GDP. Data are presented as percentage of binding stimulation over GDP basal stimulation (absence of agonist). Mean ± SEM from four separated experiments carried out in duplicate.

G-protein (Hensler, 2002, 2003; Pejchal et al., 2002; Gould and Manji, 2002; Castro et al., 2008). Recently, Czysz et al. (2015) showed that antidepressants, like S-citalopram, but not the inactive isomer R-citalopram, induce translocation of the subunit Gα out of lipid rafts.

Regarding the *in vivo* tests, the FST is a good screening tool, useful as a first approach for assessing antidepressant activity (Petit-Demouliere et al., 2005). At a first glance, the activity of the *Hypericum* extracts and their main phloroglucinols on FST seems not to be directly related to the [^{35}S]-GTPγS results, since despite

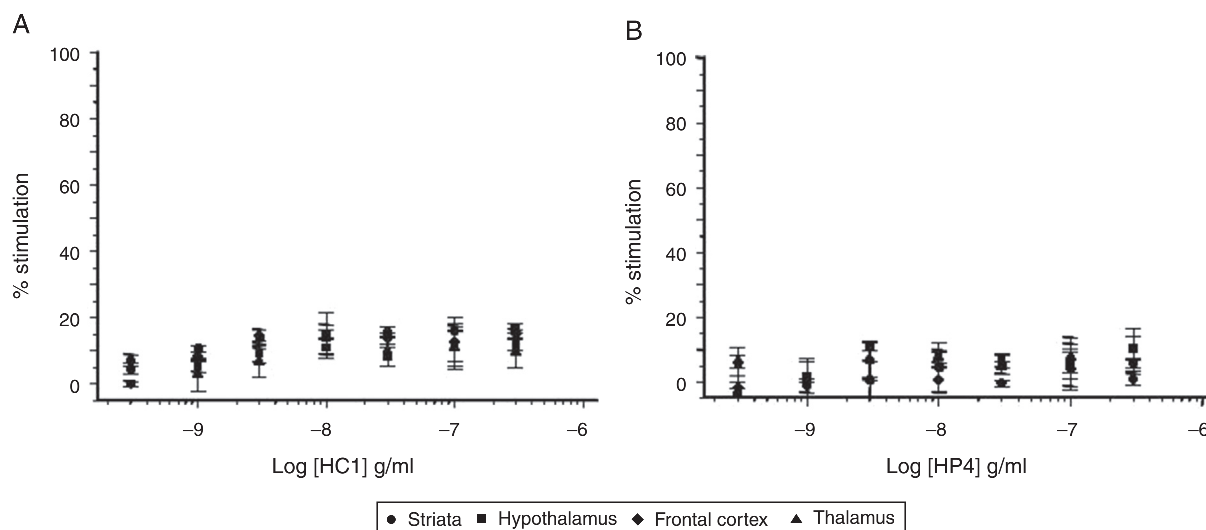

Fig. 7. Concentration–response effect of HC1 (A) and HP (B) on [^{35}S]-GTPγS binding. The data are presented as percentage of binding stimulation over GDP basal stimulation. Mean ± SEM from four separated experiments carried out in duplicate.

being effective on FST, direct incubation of membranes with HC1 and uliginosin B without agonists stimulation did not influence [^{35}S]-GTPγS binding. Therefore, the effects observed in the [^{35}S]-GTPγS binding after treating rats with POL and HCP could not be credited to an agonist effect of uliginosin B or HC1 on monoaminergic receptors. These results are in agreement with Ozawa and Rasenick (1989) and Chen and Rasenick (1995) who observed that antidepressant drugs did not have direct effect on adenylyl cyclase, a protein modulated by Gα subunit. In addition, antidepressants that acutely reduce immobility time in the FST, normally show their effects on GPCR only after 14–21 days of treatment (Hensler, 2002; Pejchal et al., 2002; Shen et al., 2002; Castro et al., 2003). Thus, it is reasonable to consider that the effects of acute and the 5 day treatments could be a consequence of monoamines increase in the synaptic cleft following the uptake inhibition induced by the extracts, HC1 and uliginosin B (Viana et al., 2005; Stein et al., 2012).

Of note, the lack of influence over DAMGO-stimulated [^{35}S]-GTPγS binding curves supports the proposition that the extracts have a selective effect on monoaminergic system. This result also corroborates a previous hypothesis that the extracts present antinociceptive activity via indirect activation of opioid system, since this effect is prevented by naloxone, but their phloroglucinols do not inhibit [^{3}H]-naloxone binding (Stolz et al., 2012).

In conclusion, our findings indicate that, at doses for which antidepressant-like activity has been demonstrated in animal models, *H. caprifoliatum* and *H. polyanthemum* extracts bring about adaptive changes in monoamine receptors, which reinforces their antidepressant-like profile. These changes are not due to an agonist action of HC1 or HP4 on monoaminergic receptors. They are likely to be a result of monoamines increase in the synaptic cleft, in consequence of monoamines uptake inhibition. Further studies are needed to elucidate which signaling pathways are activated by these extracts and compounds, but the results so far confirm that *H. caprifoliatum* and *H. polyanthemum* and their main dimeric phloroglucinol derivatives constitute promising agents to find out new antidepressants.

Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

AFV (PhD student) and JCR (Research Engineer) performed the experiments. SMKR and JC contributed on the tests design and to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Acknowledgements

This work was supported by CAPES-COFECUB (418/03 and 656/09), Fonds Européen de Développement Régional (FEDER, Convention N° 35233) and INTERREG-TC2N. We would like to thank Prof. G.L. von Poser and her staff for providing the extracts and phloroglucinol derivatives.

References

Appell, M., Berfield, J.L., Wang, L.C., Dunn III, W.J., Chen, N., Reith, M.E.A., 2004. Structure–activity relationships for substrate recognition by the human dopamine transporter. Biochem. Pharmacol. 67, 293–302.

Avissar, S., Schereiber, G., 2006. The involvement of G proteins and regulators of receptor-G protein coupling in the pathophysiology, diagnosis and treatment of mood disorders. Clin. Chim. Acta 366, 37–47.

Castro, M.E., Diaz, A., Del Olmo, E., Pazos, A., 2003. Chronic fluoxetine induces opposite changes in G protein coupling at pre and postsynaptic 5-HT1A receptors in rat brain. Neuropharmacology 44, 93–101.

Castro, E., Díaz, A., Rodriguez-Gaztelumendi, A., Del Olmo, E., Pazos, A., 2008. WAY100635 prevents the changes induced by fluoxetine upon the 5-HT1A receptor functionality. Neuropharmacology 55, 1391–1396.

Centurião, F.B., Braga, A., Machado, F.R., Tagliari, B., Müller, L.G., Kolling, J., Poser, G., Wyse, A.T., Rates, S.M., 2014. Study of antidepressant-like activity of an enriched phloroglucinol fraction obtained from *Hypericum caprifoliatum*. Pharm. Biol. 52, 105–110.

Chatterjee, S.S., Nöldner, M., Koch, E., Erdelmeier, C., 1998. Antidepressant activity of *Hypericum perforatum* and hyperforin: the neglected possibility. Pharmacopsychiatry 31, 7–15.

Chen, J., Rasenick, M.M., 1995. Chronic antidepressant treatment facilitates G protein activation of adenylyl cyclase without altering G protein content. J. Pharmacol. Exp. Ther. 275, 509–517.

Chisari, M., Saini, D.K., Kalyanaraman, V., Gautam, N., 2007. Shuttling of G protein subunits between the plasma membrane and intracellular membranes. J. Biol. Chem. 282, 24092–24098.

Czysz, A.H., Schappi, J.M., Rasenick, M.M., 2015. Lateral diffusion of Gαs in the plasma membrane is decreased after chronic but not acute antidepressant treatment: role of lipid raft and non-raft membrane microdomains. Neuropsychopharmacology 40, 766–773.

Donati, R.J., Rasenick, M.M.G., 2003. Protein signaling and the molecular basis of antidepressant action. Life Sci. 73, 1–17.

Evans, B.A., Sato, M., Sarwar, M., Hutchinson, D.S., Summers, R.J., 2010. Ligand-directed signalling at α-adrenoceptors. Br. J. Pharmacol. 159, 1022–1038.

Ferguson, S.S.G., 2001. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol. Rev. 53, 1–24.

Gould, T.D., Manji, H.K., 2002. Signaling networks in the pathophysiology and treatment of mood disorders. J. Psychosom. Res. 53, 687–697.

Harrison, C., Traynor, J.R., 2003. The [^{35}S]-GTP'S binding assay: approaches and applications in pharmacology. Life Sci. 74, 489–508.

Hensler, J.G., 2002. Differential regulation of 5-HT$_{1A}$ receptor-G protein interactions in brain following chronic antidepressant administration. Neuropsychopharmacology 26, 565–573.

Hensler, J.G., 2003. Regulation of 5-HT1A receptor function in brain following agonist or antidepressant administration. Life Sci. 72, 1665–1682.

Hermans, E., 2003. Biochemical and pharmacological control of the multiplicity of coupling at G-protein-coupled receptors. Pharmacol. Ther. 99, 25–44.

Lohse, M.J., Hein, P., Hoffmann, C., Nikolaev, O., Vilardaga, J-.P., Bünemann, M., 2008. Kinetics of G-protein-coupled receptor signals in intact cells. Br. J. Pharmacol. 153, 124–132.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Melikian, H.E., 2004. Neurotransmitter transporter trafficking: endocytosis recycling, and regulation. Pharmacol. Ther. 104, 17–27.

Millan, M.J., 2006. Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. Pharmacol. Ther. 110, 135–370.

Müller, W.E., Singer, A., Wonnemann, M., 2001. Hyperforin-antidepressant activity by a novel mechanism of action. Pharmacopsychiatry 34, 98–102.

Nör, C., Albring, D., Ferraz, A.B.F., Schripsema, J., Pires, V., Sonnet, P., Guillaume, D., von Poser, G.L., 2004. Phloroglucinol derivatives from four *Hypericum* species belonging to the *Trigynobrathys* section. Biochem. Syst. Ecol. 32, 517–519.

O'Connor, K.A., Porrino, L.J., Davies, H.M., Childers, S.R., 2005. Time-dependent changes in receptor/G-protein coupling in rat brain following chronic monoamine transporter blockade. J. Pharmacol. Exp. Ther. 313, 510–517.

Ozawa, H., Rasenick, M.M., 1989. Coupling of the stimulatory GTP-binding protein Gs to rat synaptic membrane adenylate cyclase is enhanced subsequent to chronic antidepressant treatment. Mol. Pharmacol. 36, 803–808.

Pejchal, T., Foley, M.A., Kosofsky, B.E., Waeber, C., 2002. Chronic fluoxetine treatment selectively uncouples raphe 5-HT(1A) receptors as measured by [^{35}S]-GTPγS autoradiography. Br. J. Pharmacol. 135, 1115–1122.

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

Porsolt, R.D., Bertin, A., Jalfre, M., 1977. Behavioral despair in mice: a primary screening test for antidepressants. Arch. Int. Pharmacodyn. Ther. 229, 327–336.

Porsolt, R.D., Anton, G., Blavet, N., Jalfre, M., 1978. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47, 379–391.

Rates, S.M.K., 2001. Plants as source of drugs. Toxicon 39, 603–613.

Rollinger, J.M., Langer, T., Stuppner, H., 2006. Strategies for efficient lead structure discovery from natural products. Curr. Med. Chem. 13, 1491–1507.

Rossi, D.V., Burke, T.F., Hensler, J.G., 2008. Differential regulation of serotonin-1A receptor-stimulated [^{35}S]-GTPγS binding in the dorsal raphe nucleus by citalopram and escitalopram. Eur. J. Pharmacol. 583, 103–107.

Schreiber, G., Avissar, S., 2003. Application of G-proteins in the molecular diagnosis of psychiatric disorders. Expert. Rev. Mol. Diagn. 3, 69–80.

Shen, C., Li, H., Meller, E., 2002. Repeated treatment with antidepressants differentially alters 5-HT1A agonist-stimulated [^{35}S]-GTPγS binding in rat brain regions. Neuropharmacology 42, 1031–1038.

Stahl, S.M., 2008. Essential Psychopharmacology: Neuroscientific Basis and Practical Applications. Cambridge University Press, Cambridge.

Stein, A.C., Viana, A.F., Müller, L.G., Nunes, J.M., Stolz, E.D., do Rego, J.C., Costentin, J., von Poser, G.L., Rates, S.M.K., 2012. Uliginosin B, a phloroglucinol derivative from *Hypericum polyanthemum*: a promising new molecular pattern for the development of antidepressant drugs. Behav. Brain Res. 228, 66–73.

Stolz, E.D., Viana, A.F., Hasse, D.R., von Poser, G.L., do Rego, J.C., Rates, S.M.K., 2012. Uliginosin B presents antinociceptive effect mediated by dopaminergic and opioid systems in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 39, 80–87.
Stolz, E.D., Hasse, D.R., von Poser, G.L., Rates, S.M., 2014a. Uliginosin B, a natural phloroglucinol derivative, presents a multimediated antinociceptive effect in mice. J. Pharm. Pharmacol. 66, 1774–1785.
Stolz, E.D., Müller, L.G., Antonio, C.B., da Costa, P.F., von Poser, G.L., Noël, F., Rates, S.M., 2014b. Determination of pharmacological interactions of uliginosin B a natural phloroglucinol derivative, with amitriptyline, clonidine and morphine by isobolographic analysis. Phytomedicine 21, 1684–1688.
Viana, A.F., Heckler, A.P., Fenner, R., Rates, S.M.K., 2003. Antinociceptive activity of *Hypericum caprifoliatum* and *Hypericum polyanthemum* (Guttiferae). Braz. J. Med. Biol. Res. 36, 631–634.
Viana, A.F., do Rego, J.C., von Poser, G., Ferraz, A., Heckler, A.P., Costentin, J., Rates, S.M.K., 2005. The antidepressant like effect of *Hypericum caprifoliatum* Cham & Schlecht (Guttiferae) on forced swimming test results from an inhibition of neuronal monoamine uptake. Neuropharmacology 49, 1042–1052.
Vidal, R., Valdizan, E.M., Vilaró, M.T., Pazos, A., Castro, E., 2010. Reduced signal transduction by 5-HT4 receptors after long-term venlafaxine treatment in rats. Br. J. Pharmacol. 161, 695–706.