(+)-Catharanthine and (-)-18-methoxycoronaridine induce antidepressant-like activity in mice by differently recruiting serotonergic and norepinephrinergic neurotransmission

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Running title: Antidepressant activity of coronaridine congeners

Abbreviations: (-)-18-MC, (-)-18-methoxycoronaridine; (+)-catharanthine, (+)-3,4-didehydrocoronaridine; (-)-ibogaine, 12-methoxyibogamine; noribogaine, 12-hydroxyibogamine; CFT, 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane; MPP^+, 1-methyl-4-phenylpyridinium; IC_{50}, ligand concentration that produces 50% inhibitory response (binding or function); potentiating EC_{50}, ligand concentration that produces half-maximal potentiation response; K_i, inhibitory constant; K_D, dissociation constant; K_M, Michaelis-Menten constant; n_H, Hill coefficient; NE, norepinephrine, pCPA, p-chlorophenylalanine; DSP-4, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; $[^3H]5\text{-}HT$, $[^3H]$-serotonin; $I_{5\text{-}HT,}$ inward current; 5-HT, serotonin.

Keywords: Coronaridine congeners; Antidepressant activity; mice; Monoamine transporters; serotonin receptor 3.

Abstract

The antidepressant-like activity of (+)-catharanthine and (-)-18-methoxycoronaridine [(-)-18-MC] was studied in male and female mice using forced swim (FST) and tail suspension tests (TST). The underlying molecular mechanism was assessed by electrophysiological, radioligand, and functional experiments. The FST results showed that acute administration (40 mg/kg) of (+)-catharanthine or (-)-18-MC induces similar antidepressant-like activity in male and female mice at 1 h and 24 h, whereas the TST results showed a lower effect for (-)-18-MC at 24 h. Repeated treatment at lower dose (20 mg/kg) augmented the efficacy of both congeners. The FST results showed that (-)-18-MC reduces immobility and increases swimming times without changing climbing behavior, whereas (+)-catharanthine reduces immobility time, increases swimming times more markedly, and increases climbing behavior. To investigate the contribution of the serotonin and norepinephrine transporters in the antidepressant effects of (+)-catharanthine and (-)-18-MC, we conducted in vitro radioligand and functional studies. Results obtained demonstrated that (+)-catharanthine inhibits norepinephrine transporter with higher potency/affinity than that for (-)-18-MC, whereas both congeners inhibit serotonin transporter with similar potency/affinity. Moreover, whereas no congener activated/inhibited/potentiated the function of serotonin receptor 3A or serotonin receptor 3AB, both increased serotonin receptor 3A receptor desensitization. Depletion of serotonin decreased the antidepressant-like activity of both congeners, whereas norepinephrine depletion only decreased (+)-catharanthine's activity. Our study shows that coronaridine congeners induce antidepressant-like activity in a dose- and time-dependent, and sex-independent, manner. The antidepressant-like property of both compounds involves serotonin transporter inhibition, without directly activating/inhibiting serotonin receptors 3, while (+)-catharanthine also mobilizes norepinephrinergic neurotransmission.

Introduction

Plant alkaloids such as (-)-ibogaine [and its main metabolite noribogaine (12-hydroxyibogamine)] and (+)-catharanthine, and the synthetic derivative 18-methoxycoronaridine (18-MC) are coronaridine congeners with very interesting behavioral profile, including anti-addictive (Carnicella et al., 2010; Glick et al., 1999; Glick et al., 1991; Luz,Mash, 2021; Maisonneuve,Glick, 2003), sedative (Arias et al., 2020a), and antinociceptive (Arias et al., 2020b) activities.

Previous studies showed that coronaridine congeners induce antidepressant-like activity in rats, with a concomitant increase in the brain-derived neurotrophic factor in different brain areas (Rodrıguez et al., 2020). However, the primary target(s) and exact molecular mechanism(s) involved in this activity have not been resolved yet. A plausible hypothesis is that the antidepressant-like activity of coronaridine congeners might be mediated by habenular α3β4 nicotinic acetylcholine receptors, the proposed target for their anti-addictive activity (Arias et al., 2017; Arias et al., 2015; Glick et al., 2011; Maisonneuve,Glick, 2003). However, radioligand binding studies showed that coronaridine congeners also bind to a variety of receptors and monoamine transporters with relatively high affinity (Glick,Maisonneuve, 1999; Jacobs et al., 2007). Among them, it is of note the interaction with monoamine transporters and serotonin 3 subtype receptors, whose inhibition might convey antidepressant-like activity (Amidfar,Kim, 2018).

In this regard, we want to compare the antidepressant-like activity between (-)-18-MC and (+)-catharanthine (Fig. 1) in male and female mice, after acute and repeated treatments, using both forced swim (FST) (Cryan et al., 2002) and tail suspension (TST) tests (Cryan et al., 2005a). To determine the underlying mechanisms, additional electrophysiological, radioligand, and functional experiments were undergone. More specifically, the activity of (+)-catharanthine and (-)-18-MC was determined at human serotonin receptors 3A and serotonin receptors 3AB, and human monoamine transporters, including serotonin (5-HT, serotonin transporter), dopamine (dopamine transporter), and norepinephrine (NE,

norepinephrine transporter) transporters. To assess the role of serotonergic and/or norepinephrinergic neurotransmission in the observed antidepressant-like activity, p-chlorophenylalanine (pCPA) (Zomkowski et al., 2004) and N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) (Mineur et al., 2018) were used to deplete 5-HT and NE levels, respectively.

The main conclusion of this work is that (+)-catharanthine and (-)-18-MC induce antidepressant-like activity in mice in a dose- and time-dependent, but sex-independent, manner. Their mechanism involved differential inhibition of serotonin transporter and norepinephrine transporter, with both congeners activating serotonergic transmission while norepinephrinergic pathways were affected by (+)-catharanthine only.

(-)-18-MC   (+)-Catharanthine   (-)-Ibogaine
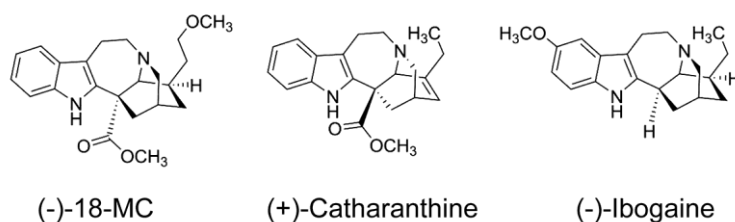
Figure 1: Molecular structures of (−)-18-methoxycoronaridine [(−)-18-MC], (+)-catharanthine [(+)-3,4-didehydrocoronaridine] and (-)-ibogaine (12-methoxyibogamine).

2. Material and Methods

2.1. Material

[³H]CFT [2β-carbomethoxy-3β-(4-fluorophenyl)-tropane] (82.6 Ci/mmol), [³H]nisoxetine (79.2 Ci/mmol), [³H]citalopram (80 Ci/mmol), [³H]dopamine (100 Ci/mmol), [³H]MPP⁺ (1-methyl-4-phenylpyridinium) (50 Ci/mmol), and [³H]5-HT (100 Ci/mmol) were purchased from Perkin Elmer New

England Nuclear (Waltham, Massachusetts, USA). Vanorexine, desipramine, paroxetine, para-chlorophenylalanine (pCPA), N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), dimethyl sulfoxide (DMSO), Tween 80, serotonin hydrochloride, and (+)-catharanthine (free base) were purchased from Sigma-Aldrich (St. Louis, MO, USA; and Saint Quentin Fallavier, France). Dulbecco's modified Eagle medium (DMEM), GlutaMAX®, NEM (non-essential amino acids), penicillin, and streptomycin were obtained from ThermoFisher (Waltham, Massachusetts, USA). (-)-18-Methoxycoronaridine hydrochloride [(-)-18-MC] was purchased from Obiter Research, LLC (Champaign, IL, USA). (+)-Catharanthine (free base) was also obtained from Henan Tianfu Chemical Co. (Zhengzhou, China). Human serotonin receptor 3A (Genebank number: BC004453) and serotonin receptor 3B (Genebank number: NM_020274) subunit cDNAs were obtained from OriGene (Rockville, MD, USA). Salts, solvents, and reagents were purchased from commercial suppliers and used as received.

2.2. Behavioral Experiments

2.2.1. Animals

All experimental procedures in mice were carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals, approved by the Regional Ethical Committee for Animal Experimentation and performed according to the European Communities Council Directive (86/609/EEC + 2010/63/UE), and by the Institutional Animal Care Committee from each institution.

Adult male and female Swiss albino CD1 mice (30-35 g), purchased from Janvier Labs (Le Genest Saint Isle, France), were used in the behavioral studies. Animals were housed in Makrolon cages (L: 37 cm, W: 21 cm, H: 14 cm), with free access to a standard semisynthetic laboratory diet (SERLAB, Montataire, France). All animals were kept in a ventilated room, at a temperature of 22 ± 1 °C, under a 12-h light/12-h dark cycle (light between 7:00 a.m. and 7:00 p.m.).

Female *Xenopus laevis* frogs were obtained from the Korean Xenopus Resource Center for Research (KXRCR000001, Chuncheon, Gangwondo, Korea). Oocytes were harvested (Lee et al., 2022) following the policy of the Laboratory Animal Ethics Committee and regulations related to animal testing (CNU IACUC-YB-2016-07).

2.2.2. Forced swim test

The forced swim test (FST) has become one of the most commonly used behavioral tests for investigating the antidepressant-like activity of drugs in rodents (Cryan,Markou, 2002). The parameters considered during the FST include three prevailing behaviors: **(a)** swimming: upon water immersion, rodents adopt an initial intense escaping behavior such as swimming and climbing. The rodent initially swims with horizontal movements across the entire swimming container after which they stop struggling and exhibit more passive behavior; **(b)** immobility: the rodent floats in the water without no struggling and moves only as much as necessary to keep its head above water. This behavior is thought to reflect either the inability to maintain an escape behavior after the stress episode (behavioral despair) or the installation of a passive behavior that prevents the animal from reaching an active state of adapting to stress (Cryan,Markou, 2002); and **(c)** climbing: the rodent delivers vigorous paw strikes to the side of the cylinder while raising the paws over the water's surface (Lino-de-Oliveira et al., 2005). A score of 1 was assigned for each climbing movement accomplished. Climbing behavior is believed to reflect more precisely the antidepressant-like effect of a drug than swimming behavior (Perona et al., 2008).

Male and female mice (n = 10/group) were habituated to the experimental room 24 h before the experiments. On the day of the experiment, mice were treated (i.p.) with 40 mg/kg (+)-catharanthine or (-)-18-MC (dissolved in 1% DMSO, 1% Tween 80, 0.9% NaCl), or vehicle. The FST was performed 1 h and 24 h after drug treatment. In addition, mice (n = 10/group) were injected (i.p.) 20 mg/kg (+)-

catharanthine or (-)-18-MC, or vehicle, for 14 consecutive days. The FST was performed 1 h after the last injection on day 7 (D_7) and day 14 (D_{14}), respectively. The doses have been chosen based on previous studies showing antidepressant-like activity of (-)-ibogaine and noribogaine (Rodrıguez,Urbanavicius, 2020), and a lack of sedative activity of (+)-catharanthine. Note that animals receiving (+)-catharanthine (Arias,Do Rego, 2020a) or (-)-18-MC, at doses of 20 or 40 mg/kg, showed locomotor activity, assessed 1 hour after their administration, similar to that observed in untreated animals, thus ruling out a possible impact of the locomotor effects of these compounds at the different doses used in the behavioral tests conducted in this study.

Each animal was placed in a cylindrical Plexiglas tank (45 cm height x 22 cm diameter) with enough water (30 cm; marked on the tank to ensure that the water volume is constant throughout the experiment; maintained at 25 °C) to preclude mice touching the bottom of the tank. After 6 min-test, animals were initially dried with paper towels, placed in a temporary dry cage with fresh bedding until completely dried, and finally returned to their original cage. The water was changed after each session to prevent any impact on the succeeding mouse.

2.2.3. Tail suspension test

The tail suspension test (TST) is another behavioral test used to screen for agents with potential antidepressant-like effects (Cryan,Mombereau, 2005a). The test consists of suspending the mouse by its tail ensuring a position where it cannot escape or cling to nearby surfaces or engage in tail-climbing behaviors. Different behaviors were analyzed by TST, including **(a)** immobility: once the mouse is suspended, the absence of escaping behavior is defined as immobility, and the concomitant immobility time measured: the shorter the immobile time, the higher the antidepressant-like activity of the drug; **(b)** lateral swing: during early mobility episodes, the rodent swings in a pendulum-like motion to gain

momentum while trying to reach its tail. A score of 1 was assigned for each lateral swing accomplished; **(c)** running movement: the rodent tries to escape by running-like movements with the front legs (Steru et al., 1985) . A score of 1 was assigned to each running movement accomplished. The increase in running movement is compensated by a decrease in lateral swing behavior. Running movement and climbing (determined by FST) are connected behaviors that respond to the same escape mechanism (Lino-de-Oliveira,De Lima, 2005); Steru,Chermat (1985); and **(d)** tail-climbing: the rodent tries to reach its tail by climbing on the horizontal bar. Since this behavior is common to both untreated and treated animals and difficult to quantify (Cryan et al., 2003), it was not further considered to assess antidepressant activity. Since mice showing high tail-climbing predisposition may produce invalid data in the TST (Mayorga,Lucki, 2001).

Male and female mice were habituated to the experimental room 24 h before the experiments. On the day of the experiment, mice (n = 14/condition) were injected (i.p.) 40 mg/kg (+)-catharanthine or (-)-18-MC (dissolved in 1% DMSO, 1% Tween 80, 0.9% NaCl), or vehicle. The TST was performed after 1 h and 24 h drug/vehicle injection. In addition, mice (n = 10/condition) were injected (i.p.) 20 mg/kg (+)-catharanthine or (-)-18-MC, or vehicle, for 14 consecutive days. The TST was performed 1 h after the last injection on D_7 and D_{14}, respectively.

For the TST, each mouse was suspended by its tail with adhesive tape, in the middle of the tail suspension box (50 height x 50 width x 14 cm depth) to impede any contact with the walls. A removable aluminum tray, placed at the bottom of the compartment, was used to collect feces/urine from mice. Precautions were made so mice do not observe other animals being tested.

2.3. Effect of pCPA on the antidepressant-like activity of (+)-catharanthine and (-)-18-MC

To assess the involvement of serotonergic neurotransmission in the antidepressant-like activity of (+)-catharanthine and (-)-18-MC, male mice (n = 10/condition) were administered (i.p.) 150 mg/kg pCPA or vehicle (saline solution), daily for three consecutive days. pCPA is a selective inhibitor of 5-HT biosynthesis that induces 85–95% depletion of the endogenous 5-HT pool while not impacting the respective content of NE and dopamine in this regimen (Eckeli et al., 2000; Zomkowski,Rosa, 2004). On the fourth day, mice were treated with each congener (40 mg/kg; i.p.) separately and the FSTs performed at 1 h and 24 h.

2.4. Effect of DSP-4 on the antidepressant-like activity of (+)-catharanthine and (-)-18-MC

To assess the involvement of norepinephrinergic neurotransmission in the antidepressant-like activity of (+)-catharanthine and (-)-18-MC, male mice (n = 10/condition) were administered (i.p.) a single dose of 50 mg/kg DSP-4, or vehicle (saline solution). DSP-4 is a neurotoxin that selectively induces norepinephrinergic denervation in the locus coeruleus and subsequent NE depletion (Mineur,Cahuzac, 2018). We chose to use this dose in accordance with previous studies showing tissue depletion of NE in the brain (Ross,Stenfors, 2015). Three days after treatment with DSP-4, mice were injected each congener (40 mg/kg; i.p.) separately, and the FSTs performed at 1 h and 24 h, respectively.

2.5. Effect of coronaridine congeners on monoamine transporters

2.5.1. Radioligand binding experiments

HEK 293 cells stably expressing the human transporters for dopamine (dopamine transporter/SLC6A3), norepinephrine (norepinephrine transporter/SLC6A2), and serotonin (serotonin transporter/SLC6A4), were grown in Dulbecco's Modified Eagle Media (DMEM) supplemented with 10% heat-inactivated fetal

calf serum (FBS), 0.6 mg/L penicillin, 0.1 g/L streptomycin and 0.1 g/L geneticin/G418 (to maintain selective pressure). Confluent monolayers of cells were mechanically detached and lysed in hypotonic buffer (20 mM Tris.HCl, pH 7.4, 2 mM EDTA, 2 mM $MgCl_2$; EDTA was omitted for dopamine transporter expressing cells). The cell suspension was frozen in liquid nitrogen, subjected to freeze-thaw cycles and sonicated. The homogenate was finally centrifuged at 30,000 g for 30 min at 4 °C. The resulting pellet was resuspended at a protein concentration of about 5 mg/ml, aliquoted, frozen in liquid nitrogen, and stored at -80 °C.

Binding assays were performed in a final volume of 0.1 mL, containing membranes (2 to 4 µg) suspended in buffer (20 mM Tris.HCl, pH 7.4, 1 mM EDTA, 2 mM $MgCl_2$, 120 mM NaCl, 3 mM KCl; EDTA was replaced with 10 µM $ZnCl_2$ for assays with membranes harboring dopamine transporter, logarithmically spaced concentrations (0.3–300 µM) of (-)-ibogaine, (+)-catharanthine, or 18-MC, and the radioligand (i.e., 7 nM [^{3}H]CFT, 3 nM [^{3}H]nisoxetine, and 2 nM [^{3}H]citalopram) for assessing binding to the dopamine transporter, norepinephrine transporter, and the serotonin transporter, respectively. The incubation was performed for 60 minutes followed by rapid filtration over GF/B filters, which had been presoaked in 0.5% polyethyleneimine. Nonspecific binding, assessed in the presence of 10 µM vanorexine (dopamine transporter), 10 µM desipramine (norepinephrine transporter), and 10 µM paroxetine (serotonin transporter), was <10 % of total binding.

2.5.2. Inhibition of substrate uptake

HEK293 cells stably expressing the human dopamine transporter, norepinephrine transporter or serotonin transporter were seeded (~2x10^4 cells/well) into poly-D-lysine-coated 96-well plates. After 24 h, the medium in each well was aspirated and the cells were washed once with Krebs-HEPES buffer (10 mM HEPES.NaOH, pH 7.4, 120 mM NaCl, 3 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, and 2 mM glucose). Cells

were pre-incubated in a buffer containing logarithmically spaced concentrations (0.3–300 µM) of each coronaridine congener for 10 min. The uptake reaction was started by the addition of the respective substrate [i.e., 0.1 µM [^{3}H]dopamine (dopamine transporter), 0.1 µM [^{3}H]5-HT (serotonin transporter), or 0.05 µM [^{3}H]MPP$^+$ (norepinephrine transporter)]. After 1 min, the reaction was terminated by aspirating the medium followed by a wash with ice-cold buffer. Cells were lysed with 1% SDS to release the accumulated radioactive substrate. The released radioactivity was quantified by liquid scintillation counting. Non-specific uptake, determined in the presence of 10 µM vanorexine (dopamine transporter), 100 µM desipramine (norepinephrine transporter), or 10 µM paroxetine (serotonin transporter), was <5 % of total uptake.

Radioligand binding and uptake inhibition data were subjected to a non-linear, least-squares curve fitting to the equation for a monophasic inhibition to obtain IC_{50} estimates. Since the substrates were used at very low concentrations (i.e., $<0.1*K_M$), the IC_{50} estimates approximate the K_i (i.e., binding affinity) values.

2.6. Effect of coronaridine congeners on human serotonin receptor 3A and serotonin receptors 3AB using two-electrode voltage-clamp

The cDNA of each human serotonin receptor 3A and serotonin receptor 3B subunit was linearized by Xho I and Pme I at the end of the multi-cloning site, followed by transcription using the mMESSAGE mMACHINE T7 Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA), as described previously (Lee,Seol, 2022). The mRNA products were dissolved in diethyl pyrocarbonate and stored at -80°C until use. To express the respective homopentamer serotonin receptor 3A and heteropentamer 5 serotonin receptors 3B, 50 ng serotonin receptor 3A mRNA or 25 ng serotonin receptor 3A mRNA:25 ng serotonin receptor 3B mRNA was injected per oocyte. After two days, the activity of each receptor

subtype was recorded using a two-electrode voltage clamp (Warner Instruments, Hamden, CT, USA), subsequently digitalized (Digidata, Molecular Devices, Sunnyvale, CA, USA), and analyzed using the pClamp 10 software (Axon Instruments, Union City, CA, USA). The voltage and current electrodes were filled with 3 M KCl (0.3–0.7 MW) and analyzed at −70 mV holding potential. Oocytes were placed in the chamber and exposed to an ND96 solution at a flow rate of 2 mL/min. Inward peak traces elicited by 100 μM 5-HT, in the absence and presence of each congener (drugs were dissolved in 0.1% DMSO), were analyzed using Clampfit 9.0 (Molecular Devices, San Jose, CA, USA). The deactivation recovery time of 100 μM 5-HT   was determined by measuring the time of the deactivation process from maximal (peak; 100%) to basal (0%) 5-HT -evoked currents (Solt et al., 2007). Dopamine transporter charge (i.e., current amplitude area) was normalized to that for 5-HT alone as previously described (Papke,Porter Papke, 2002).

2.7. Statistical Analysis

Experimental data (mean ± SEM) were analyzed by using the Prism software (GraphPad 6.0 or 8.4.1., Software Inc., La Jolla, CA, USA). Two-way ANOVA analysis was used to compare drug type-, dose-, and time treatments in the behavioral studies. Tukey's multiple comparison post-hoc test was used to compare different sexes and acute vs repeated treatments. One-way ANOVA analysis was used to compare the effect of 5-HT in the absence and presence of coronaridine congeners. Values of $p < 0.05$ were considered significantly different.

3. Results

3.1. (+)-Catharanthine and (-)-18-MC increase both swimming time and climbing behavior, and decrease immobility time, in the FST after one, seven, or fourteen days of treatments

The antidepressant-like activity of (+)-catharanthine and (-)-18-MC was determined after acute (1 h and 24 h) and repeated (D_7 and D_{14}) treatments using FST. This test allowed us to assess different escape behaviors, including swimming, immobility, and climbing (Fig. 2A) (see Methods section for more details).

Two-way ANOVA and Tukey's post-hoc analyses showed that acute treatment with (+)-catharanthine or (-)-18-MC significantly increased swimming time (Fig. 2B) and decreased immobility time (Fig. 2C) after 1 h and 24 h post-injection with similar statistical values for both congeners [$F_{(4, 90)}$ = 88.58; $p < 0.0001$], compared to vehicle-treated animals. The analyses showed no significant difference between 1 h and 24 h for swimming [$F_{(4, 90)}$ = 0.1305; $p = 0.9709$] (Fig. 2B) and immobility [$F_{(4, 90)}$ = 88.58; $p = 0.3799$] times (Fig. 2C), and between male and female mice [$F_{(4, 90)}$ = 0.1305; $p = 0.97$; for both behaviors] (Table 1).

A lower dose (20 mg/kg) of (+)-catharanthine or (-)-18-MC was able to increase swimming time (Fig. 2E) and decrease immobility time (Fig. 2F) at D_7 or D_{14} ([$F_{(5, 108)}$ = 209.4; $p < 0.0001$]; for both), compared to vehicle-treated animals (Table 1). No significant differences were reported between males and females in the swimming [$F_{(1, 108)}$ = 0.00048; $p = 0.982$] (Fig. 2E) and immobility [$F_{(1, 108)}$ = 0.08797; $p = 0.954$] (Fig. 2F) behaviors, between acute and D_7 [$F_{(3, 72)}$ = 371; $p = 0.9999$] or D_{14} [$F_{(3, 72)}$ = 371; $p = 0.608$], and between D_7 and D_{14} [$F_{(5, 108)}$ = 209; $p = 0.877$] for each behavior.
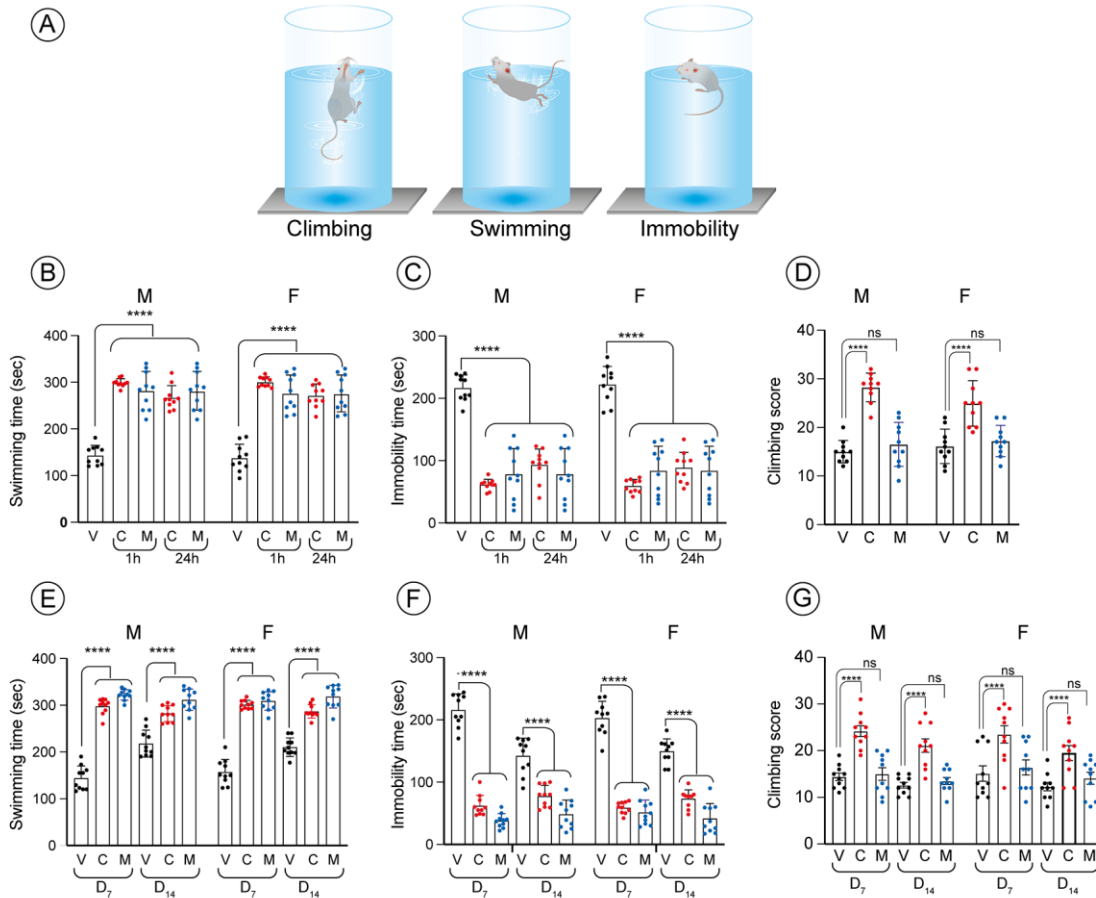
Figure 2. Antidepressant-like activity of (+)-catharanthine and (-)-18-MC after acute and repeated treatments determined by FST. (A) FSTs allowed us to study different escape behaviors, including swimming, immobility and climbing. (B-D) Acute treatment: male and female mice (n = 10/condition) were injected (i.p.) 40 mg/kg (+)-catharanthine (C) or (-)-18-MC (M), or vehicle (V), and FSTs performed after 1 h and 24 h, respectively. (E-G) Repeated treatment: male and female mice (n = 10/condition) were injected (i.p.) 20 mg/kg (+)-catharanthine or (-)-18-MC, or vehicle, for 14 consecutive days, and FSTs were performed 1 h after the last injection on day 7 (D_7) and day 14 (D_{14}), respectively. Two-way ANOVA and post hoc Turkey's analyses gave the following results: ns, not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

The effect of (+)-catharanthine and (-)-18-MC on climbing behavior was also studied after acute (40 mg/kg) (Fig. 2C) and repeated treatments (20 mg/kg) (Fig. 2G). Statistical analyses of the acute treatment showed that (+)-catharanthine [$F_{(2, 54)} = 54.69$; $p < 0.0001$], but not (-)-18-MC [$F_{(5, 108)} = 22.17$; $p = 0.9389$], significantly increases climbing, compared to vehicle-treated animals, with no statistical difference between males and females [$F_{(1, 108)} = 0.00048$; $p = 0.9825$] (Fig. 2D) (Table 1).

Statistical analyses of the effect of (+)-catharanthine and (-)-18-MC on climbing behavior after repeated treatment showed a significant effect for (+)-catharanthine at D_7 [$F_{(5, 108)} = 22.17$; $p < 0.0001$] and D_{14} [$F_{(5, 108)} = 22.17$; $p = 0.0007$] (20 mg/kg), but not for (-)-18-MC [$F_{(5, 108)} = 22.17$; $p = 0.9999$; at D_7 and D_{14}], compared to vehicle-treated animals (Table 1). (+)-Catharanthine's effects were similar between males and females [$F_{(1, 62)} = 0.0686$; $p = 0.7937$], between D_7 and D_{14} [$F_{(8, 162)} = 28.94$; $p0.999$], and between acute and D_7 [$F_{(3, 72)} = 387.5$; $p = 0.999$] or D_{14} [$F_{(3, 72)} = 370.8$; $p = 0.6078$] treatments. In summary, (+)-catharanthine, but not (-)-18-MC, induces escape behavior, in a dose- and time-dependent, but sex-independent, manner.

3.3. (+)-Catharanthine and (-)-18-MC reduce both immobility and lateral swing behaviors and increase running behavior in the TST after acute and repeated treatments

The antidepressant-like activity of (+)-catharanthine and (-)-18-MC was also studied in male and female mice after acute (1 h and 24 h), and prolonged (D_7 and D_{14}) treatments by TST. This test was used to support the initial results obtained by FSTs, and to evaluate distinct escape behaviors, including immobility, lateral swing, and running behaviors (Fig. 3A) (see Methods section for more details). The results of the running behavior in TST usually parallel the results of climbing behavior in FST with the same escape mechanism (Lino-de-Oliveira,De Lima, 2005; Steru,Chermat, 1985), and both parameters can be used to assess drug-induced antidepressant-like mechanisms.

Two-way ANOVA and Tukey's post-hoc analyses of the acute results showed that 40 mg/kg (+)-catharanthine significantly reduces immobility time in both male and female mice at 1 h and 24 post-injection compared to vehicle-treated animals ([$F_{(4, 90)} = 84.06$; $p < 0.0001$]), whereas 40 mg/kg (-)-18-MC showed significant effect at 24 h [$F_{(4, 90)} = 84.06$; $p < 0.0001$] but not at 1 h [$F_{(4,90)} = 84.06$; $p = 0.576$] (Fig. 3B). There was a significant difference between 1 h and 24 h for (-)-18-MC [$F_{(4,90)} = 84.06$; $p < 0.0001$], but not (+)-catharanthine [$F_{(4, 90)} = 84.06$; $p = 0.999$], and no difference between male and female mice for both congeners [$F_{(1, 90)} = 0.0237$; $p = 0.813$].

Statistical analyses of the acute results also showed that 40 mg/kg (+)-catharanthine significantly reduces lateral swing scores [$F_{(2, 54)} = 48.04$; $p < 0.0001$] (Fig. 3C) and significantly increases running scores [$F_{(2, 54)} = 469.9$; $p < 0.0001$] (Fig. 3D), compared to vehicle-treated animals, whereas (-)-18-MC affected neither running [$F_{(2,54)} = 469.9$; $p = 0.971$] nor lateral swing [$F_{(2,54)} = 1.297$; $p = 0.999$] behaviors (Table 1). (+)-Catharanthine's effects were similar between males and females on lateral swing scores [$F_{(1, 54)} = 0.308$; $p = 0.945$] (Fig. 3C) and running scores [$F_{(1, 54)} = 0.1006$; $p = 0.999$].

Treated animals alternated between swinging and running movements for longer periods compared to vehicle-treated animals. Vehicle-treated animals adopted a shorter swinging behavior remaining motionless afterward, whereas treated animals energetically moved their front legs to try to escape at expense of swinging movements, which explains the decrease in swing score.

Repeated treatment using a dose of 20 mg/kg of either (+)-catharanthine or (-)-18-MC showed a similar trend as that reported in the acute treatment using a dose of 40 mg/kg (Fig. 3E). Two-way ANOVA and Tukey's post-hoc analyses showed a significant decrease in immobility time for (+)-catharanthine [$F_{(5, 108)} = 103.4$; $p < 0.0001$] and (-)-18-MC [$F_{(5, 108)} = 103.4$; $p < 0.0001$] with same statistical values for D_7 and D_{14}. No significant difference between males and females at both D_7 and D_{14} was reported for (+)-catharanthine [$F_{(1, 108)} = 0.04364$; $p = 0.999$] and (-)-18-MC [$F_{(1, 108)} = 0.04364$; $p = 0.999$].

However, behavioral differences were observed between (+)-catharanthine and (-)-18-MC (20 mg/kg) at D_7 and D_{14}. Indeed, two-way ANOVA analyses showed no significant decrease in lateral swing score for (+)-catharanthine and (-)-18-MC [$F (5, 108) = 4.686$; $p = 0.14$; for both D_7 and D_{14}] (Fig. 3F) compared to vehicle-treated animals (Table 1), and a significant increase in running score for (+)-catharanthine [$F (5, 108) = 468.3$; $p < 0.0001$], but not (-)-18-MC [$F (5, 108) = 468.3$; $p = 0.999$] (Fig. 3G), with same statistical values at D_7 and D_{14}. No significant difference was reported between males and females for the effect of (+)-catharanthine [$F (1,108) = 1.555$; $p = 0.999$] and (-)-18-MC [$F (1,108) = 1.555$; $p = 0.999$] on lateral swing score (Fig. 3F), and for the effect of (+)-catharanthine [$F (1, 108) = 0.08819$; $p = 0.999$] on running score (Fig. 3G), for both D_7 and D_{14}.


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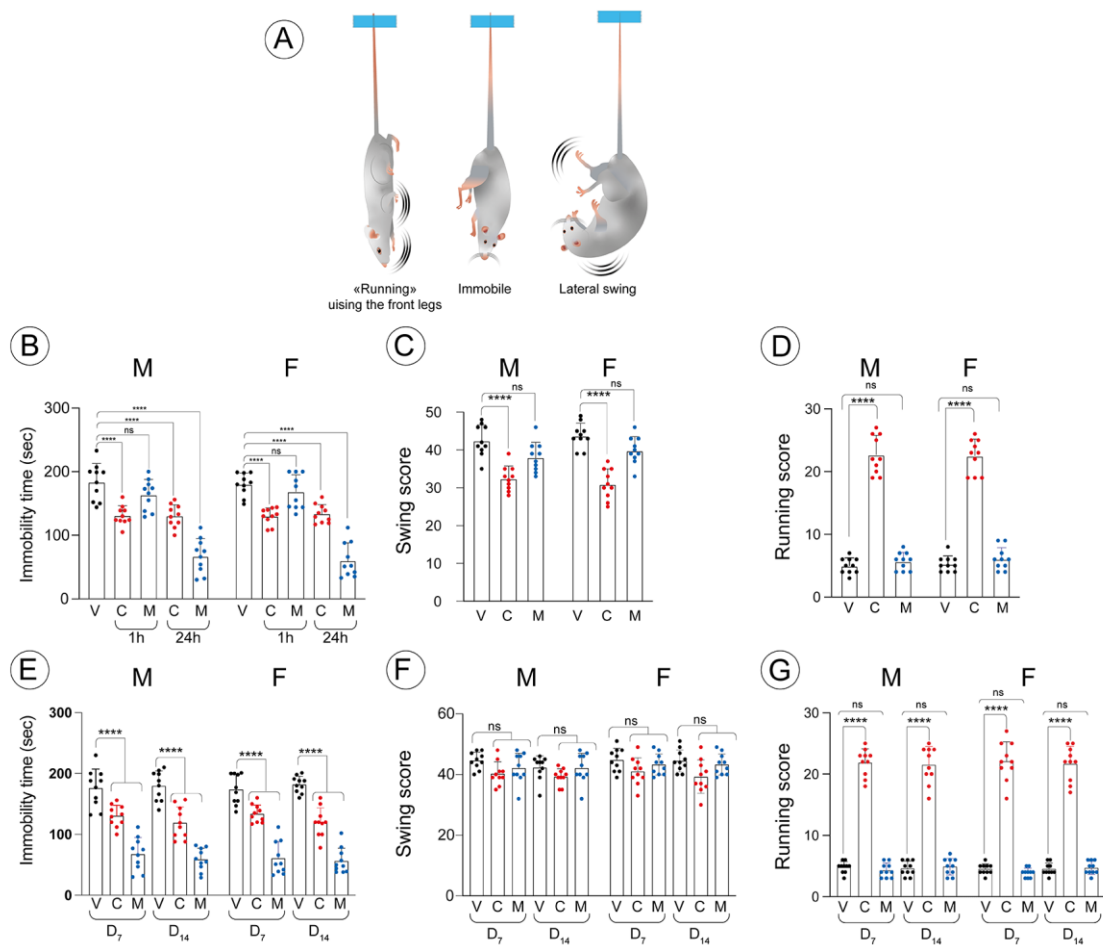
Figure 3. Antidepressant-like activity of (+)-catharanthine and (-)-18-MC after acute and repeated treatments determined by TST. (A) TSTs allowed us to study three behaviors, including immobility, lateral swing and running. (B-D) Acute treatment: male (M) and female (F) mice (n = 14/condition) were injected (i.p.) 40 mg/kg (+)-catharanthine (C) or (-)-18-MC (M), or vehicle (V), and TSTs performed after 1 h and 24 h, respectively. Effect of (+)-catharanthine and (-)-18-MC on swimming time (B), lateral swing (C), and running behavior (D). (E-G) Repeated treatment: mice (n = 14/condition) were injected (i.p.) 20 mg/kg (+)-catharanthine or (-)-18-MC for 14 consecutive days, and the TSTs performed 1 h after the last injection on day 7 (D_7) and day 14 (D_{14}), respectively. Effect of (+)-catharanthine and (-)-18-MC on swimming time (E), lateral swing (F), and running behavior (G). Two-way ANOVA and post hoc Turkey's analyses gave the following results: ns, not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

3.3. Role of serotonergic neurotransmission in the antidepressant-like activity of (+)-catharanthine and (-)-18-MC

To assess the role of serotonergic neurotransmission in the antidepressant-like activity of (+)-catharanthine and (-)-18-MC, male mice were pretreated with pCPA to inhibit the endogenous synthesis of 5-HT (Eckeli,Dach, 2000; Zomkowski,Rosa, 2004). Two-way ANOVA analyses showed that pCPA did not alter swimming time *per se* compared to vehicle-treated animals [$F_{(4, 90)} = 17.57$; $p = 0.0284$]. However, pCPA pretreatment significantly reduced the increase in swimming time induced by (-)-catharanthine or (-)-18-MC at 1 h [$F_{(4, 90)} = 17.57$; $p < 0.0001$] and 24 h [$F_{(4, 90)} = 17.57$; $p = 0.0022$] (Fig. 4A), and significantly increased the decrease in immobility time with same statistical values at both times [$F_{(4, 90)} = 17.57$; $p < 0.0001$] (Fig. 4B), compared to pCPA-untreated animals. The effect of (+)-catharanthine and (-)-18-MC on climbing behavior was also studied after pretreatment with pCPA (Fig. 4C). Two-way ANOVA analyses showed that pCPA significantly decreased (+)-catharanthine's effect [F

(4, 90) = 265.4; $p < 0.0001$], without changing the previously observed lack of effect by (-)-18-MC [F (4, 90) = 265.4; $p = 0.884$], compared to pCPA-untreated animals. These results show that the activity of both congeners was sensitive to pretreatment with pCPA.
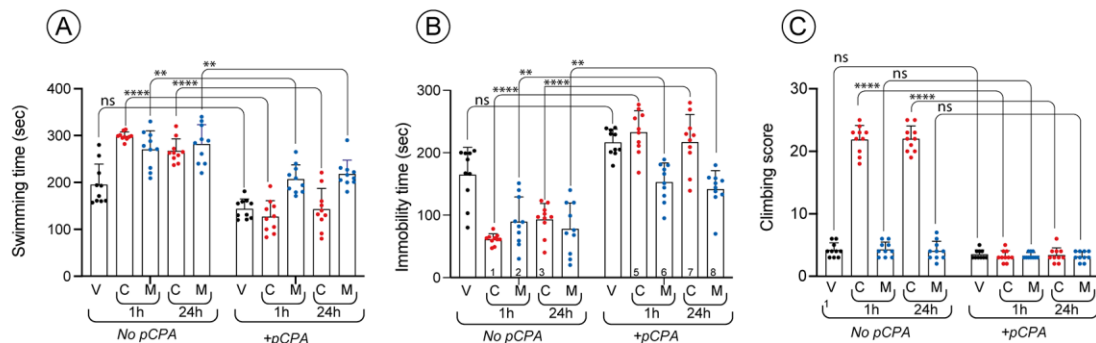
Figure 4: Effect of pCPA on the acute antidepressant-like activity of (+)-catharanthine and (-)-18-MC. Mice (n = 10/condition) were administered (i.p.) 150 mg/kg pCPA or vehicle, daily for three consecutive days. On the fourth day, animals were injected (i.p.) 40 mg/kg (+)-catharanthine (C) or (-)-18-MC (M), or vehicle (V), and FSTs performed at 1 h and 24 h, respectively. (A-C) pCPA did not modify *per se* the swimming time compared to vehicle-treated animals. However, pCPA pretreatment significantly reduced the increase in swimming time (A) and increased the decrease in immobility time (B) induced by (-)-catharanthine or (-)-18-MC at 1 h and 24 h, and reduced climbing behavior elicited by (+)-catharanthine without changing the lack of climbing behavior by (-)-18-MC (C), compared to pCPA-untreated animals. Two-way ANOVA and post hoc Turkey's analyses gave the following results: ns, not significant, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

3.4. Role of norepinephrinergic neurotransmission in the antidepressant-like activity of (+)-catharanthine and (-)-18-MC

To assess the role of norepinephrinergic neurotransmission in the antidepressant-like activity of (+)-catharanthine and (-)-18-MC, male mice were pretreated with DSP-4 to deplete the endogenous content of NE (Mineur,Cahuzac, 2018). Two-way ANOVA analyses showed that DSP-4 did not modify *per se* the swimming time compared to vehicle-treated animals [F (1, 90) = 144; p = 0.097] (Fig. 5A). However, DSP-4 pretreatment significantly reduced the increase in swimming time induced by (-)-catharanthine with same statistical values at 1 h and 24 h [F (1, 90) = 144; p < 0.0001], without affecting (-)-18-MC's activity at 1 h [F (1, 90) = 144; p = 0.805] and 24 h [F (1, 90) = 144; p = 0.394], respectively, compared to DSP-4-untreated animals. In addition, DSP-4 pretreatment significantly increased the decrease in immobility time [F (4, 90) = 27.43; p < 0.0001] (Fig. 5B) and significantly reduced climbing behavior [F (4, 90) = 119.5; p < 0.0001] (Fig. 5C) elicited by (+)-catharanthine with same statistical values at 1 h and 24 h, compared to DSP-4-untreated animals. However, DSP-4 affected neither the decrease in immobility time [F (4, 90) = 25.30; p = 0.760)], nor the lack of climbing behavior previously showed for (-)-18-MC at 1 h and 24 h [F (4, 90) = 119.5; p = 0.999] (Figs. 5A-C). These results show that only the activity of (+)-catharanthine is sensitive to DSP-4 pretreatment.
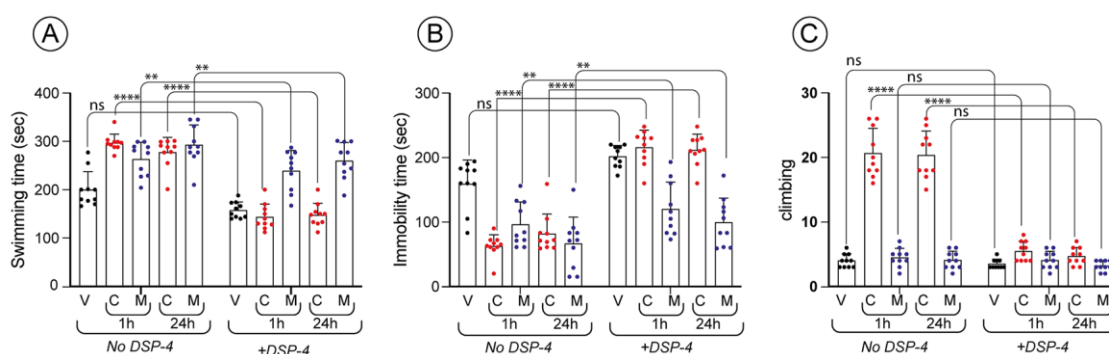
Figure 5: Effect of DSP-4 on the acute antidepressant-like activity of (+)-catharanthine and (-)-18-MC. Mice (n = 10/condition) were administered a single dose of 50 mg/kg DSP-4 (i.p.) or vehicle. Three days

after treatment with DSP-4, animals were injected (i.p.) 40 mg/kg (+)-catharanthine (C) or (-)-18-MC (M), or vehicle (V), and FSTs performed at 1 h and 24 h, respectively. (A-C) DSP-4 did not modify *per se* the swimming time compared to vehicle-treated animals. However, DSP-4 pretreatment significantly reduced the increase in swimming time (A), increased the decrease in immobility time (B), and reduced climbing behavior (C) induced by (-)-catharanthine with the same statistical values at 1 h and 24 h, without affecting (-)-18-MC's activity, compared to DSP-4-untreated animals. Two-way ANOVA and post hoc Turkey's analyses gave the following results: ns, not significant, $*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$.

Table 1. Summary of behavioral activities mediated by coronaridine congeners after acute and repeated treatments using both FST and TST.

Test	Behavior	Treatment	(+)-Catharanthine	(-)-18-MC
FST	Swimming	Acute	Increase [a]	Increase [a]
	Swimming	Acute (+pCPA)	Reduce [b]	Reduce [b]
	Swimming	Acute (+DSP-4)	Reduce [b]	No effect [b]
	Swimming	Repeated	Increase [a]	Increase [a]
	Immobility	Acute	Reduce [a]	Reduce [a]
	Immobility	Acute (+pCPA)	Increase [b]	Increase [b]
	Immobility	Acute (+DSP-4)	Increase [b]	No effect [b]
	Immobility	Repeated	Reduce [a]	Reduce [a]
	Climbing	Acute	Increase [a]	No effect [a]
	Climbing	Acute (+pCPA)	Reduce [b]	No effect [b]
	Climbing	Acute (+DSP-4)	Reduce [b]	No effect [b]
	Climbing	Repeated	Increase [a]	No effect [a]
TST	Immobility	Acute	Reduce [a]	Reduce [a]
	Immobility	Repeated	Reduce [a]	Reduce [a]

Lateral swing	Acute	Reduce [a]	No effect [a]
Lateral swing	Repeated	Reduce [a]	No effect [a]
Running	Acute	Increase [a]	No effect [a]
Running	Repeated	Increase [a]	No effect [a]

[a] Two-way ANOVA analyses showed significant effects for each congener compared to vehicle-treated animals.

[b] Two-way ANOVA analyses showed significant effects for pCPA or DSP-4 pretreatment on congener-treated mice compared to the respective pCPA (or DSP-4)-untreated animals.

3.5. Interaction of (+)-catharanthine, (-)-18-MC, and (-)-ibogaine with monoamine transporters

The chemical structure of (+)-catharanthine and (-)-18-MC is related to that of (-)-ibogaine (Fig. 1). Since (-)-ibogaine binds to the inward facing conformation of monoamine transporters (Bulling et al., 2012; Jacobs,Zhang, 2007), it is classified as an atypical inhibitor (Bhat et al., 2019). The indole ring and the tropane-based ring system, which are both present in (+)-catharanthine and (-)-18-MC, are crucial determinants for binding to serotonin transporter and dopamine transporter (Bhat et al., 2021). Accordingly, we examined the ability of (+)-catharanthine and (-)-18-MC to displace high-affinity radiolabeled inhibitors and to block substrate uptake in human dopamine transporter, norepinephrine transporter, and serotonin transporter, respectively.

The radioligand binding assays allowed us to calculate the binding affinity for each studied coronaridine congener at dopamine transporter (Fig. 6A), norepinephrine transporter (Fig. 6B), and serotonin transporter (Fig. 6C), respectively. (+)-catharanthine and (-)-18-MC have lower affinity than (-)-ibogaine for all three monoamine transporters as follows (from higher to lower affinity): (-)-ibogaine >

(+)-catharanthine > (-)-18-MC (Table 3). It is also clear that there was a substantial difference (i.e., ~10-fold) between the affinity of (+)-catharanthine and (-)-18-MC for dopamine transporter and norepinephrine transporter, whereas the difference was less pronounced for serotonin transporter (i.e., ~1.5-fold) (Table 2). The affinity of (-)-18-MC for serotonin transporter was higher than that for dopamine transporter (16-fold) and norepinephrine transporter (19-fold) (Table 2).
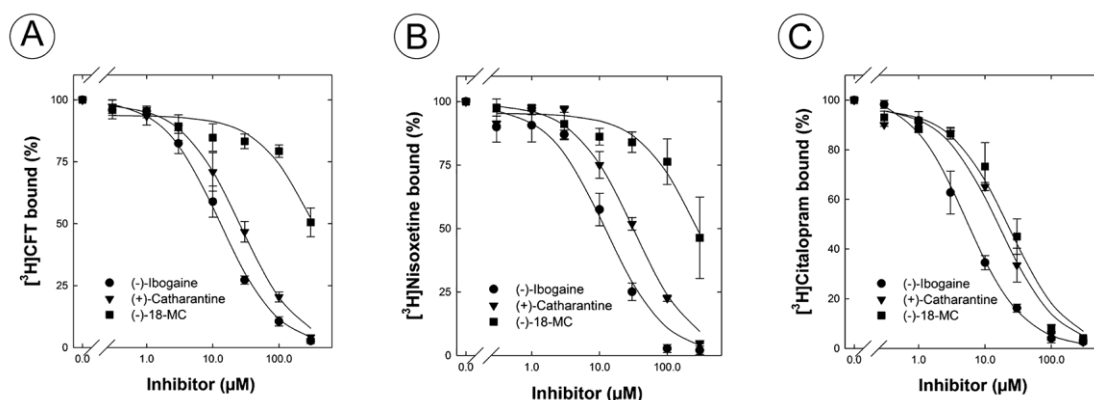
Figure 6: Effect of coronaridine congeners on radioligand binding to human dopamine (dopamine transporter) (A), norepinephrine (norepinephrine transporter) (B), and serotonin (serotonin transporter) (C) transporters. Membranes (2-4 μg/assay), prepared from HEK293 cells stably expressing dopamine transporter, norepinephrine transporter, or serotonin transporter, were incubated with 7 nM [^{3}H]CFT (A), 3 nM [3H]nisoxetine (B), or 2 nM [^{3}H]citalopram (C), and different concentrations (i.e., 0.3-300 μM) of (-)-ibogaine (●), (+)-catharanthine (▼), or (-)-18-MC (■). Nonspecific binding, assessed in the presence of 10 μM vanorexine (dopamine transporter), 10 μM desipramine (norepinephrine transporter), or 10 μM paroxetine (serotonin transporter), was <10 % of total binding. The specific binding was normalized to 100% (mean ± S.D; n = three independent experiments) and subjected to non-linear, least-squares curve fitting to the equation for a monophasic inhibition. The obtained IC$_{50}$ (i.e., binding affinity) values are summarized in Table 2.

We also independently verified that both (+)-catharanthine and (-)-18-MC were able to block substrate uptake in monoamine transporters with lower potency (i.e., higher IC$_{50}$ values) compared to that for (-)-ibogaine (Table 3). When compared to (+)-catharanthine, (-)-18-MC has a substantially lower potency at dopamine transporter (Fig. 7A) and norepinephrine transporter (Fig. 7B), and displays similar potency at the serotonin transporter (Fig. 7C) (Table 2).
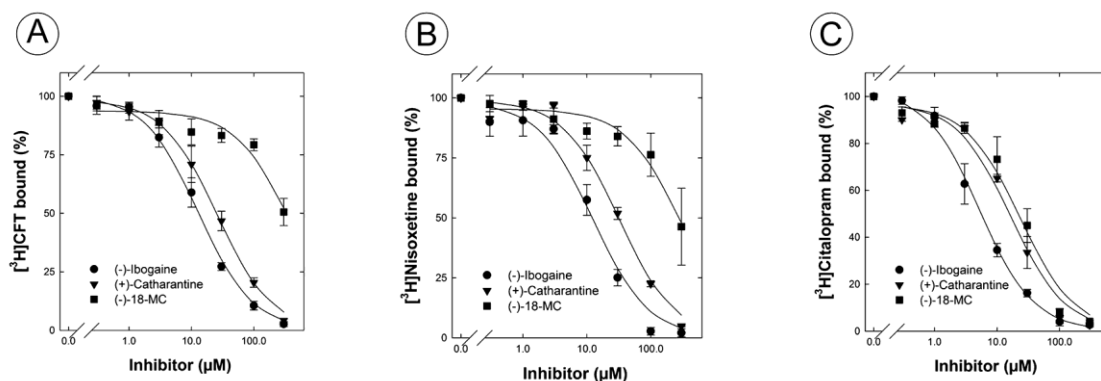
Figure 7. Effect of coronaridine congeners on substrate uptake at human dopamine transporter (A), norepinephrine transporter (B), and serotonin transporter (C). HEK293 cells ($2*10^4$/well) stably expressing dopamine transporter, norepinephrine transporter, or serotonin transporter, were preincubated with different concentrations (i.e., 0.3-300 μM) of (-)-ibogaine (●), (+)-catharanthine (▼), or (-)-18-MC (■) for 10 min. The uptake reaction was initiated by addition of 0.1 μM [^{3}H]dopamine (A), 0.05 μM [^{3}H]MPP$^+$ (B), or 0.1 μM [^{3}H]5-HT (C), and carried out for 1 min. Nonspecific uptake of [^{3}H]dopamine (A), [^{3}H]MPP$^+$ (B), and [^{3}H]5-HT(C) was determined in the presence of 10 μM vanorexine, 100 μM desipramine, and 10 μM paroxetine, respectively, and subtracted from total uptake in the absence of inhibitor to yield specific uptake. Specific uptake values (mean ± SD), in the range of 2.1 ± 0.3 (A), 3.2 ± 0.2 (B) and 5.3 ± 0.5 (C) pmol/min 10^{-6} cells, were normalized to 100%. Experimental data (mean ± S.D;

n = three independent experiments) were subjected to non-linear, least-squares curve fitting to the equation for a monophasic inhibition. The calculated IC_{50} values are summarized in Table 2.

Table 2. Pharmacological activity of coronaridine congeners at human monoamine transporters and serotonin receptors.

Monoamine Transporter	Drug	Binding Affinity [a]	Inhibitory potency [b]
		K_i (μM)	IC_{50} (μM)
dopamine transporter	(-)-Ibogaine	8.5 ± 1.6	36.5 ± 3.4
	(+)-Catharanthine	21.7 ± 2.9	82.0 ± 10.2
	(-)-18-MC	207 ± 33 [c]	1177 ± 102 [c]
norepinephrine transporter	(-)-Ibogaine	8.3 ± 0.8	28.8 ± 5.2
	(+)-Catharanthine	16.6 ± 1.4	30.7 ± 3.6
	(-)-18-MC	244 ± 48	121 ± 10
serotonin transporter	(-)-Ibogaine	2.9 ± 0.5	15.6 ± 3.5
	(+)-Catharanthine	8.4 ± 1.7	46.2 ± 12.5
	(-)-18-MC	12.6 ± 2.3	51.6 ± 9.6

[a] Data obtained from radioligand binding assays (Figs. 6A-C).

[b] Data obtained from substrate uptake assays on monoamine transporters (Figs. 7A-C)

[c] Since these values were obtained from concentration-response curves showing ≤50% inhibition, they do not intend to be accurate values but are included for comparative purposes.

3.6. (+)-Catharanthine and (-)-18-MC do not reduce serotonin-evoked serotonin receptor 3 currents but reduce deactivation recovery time only at the serotonin receptor 3A :

To determine whether coronaridine congeners modify the activity of serotonin receptors 3, electrophysiological recordings (-70 mV holding potential) were performed in *Xenopus* oocytes expressing either the serotonin receptor 3A or serotonin receptor 3AB. The first series of experiments indicated that neither (+)-catharanthine nor (-)-18-MC activate the serotonin receptor 3A (Fig. 8A) or serotonin receptor 3AB (Unpublished results) subtype. Corresponding to a higher potency of 5-HT for the serotonin receptor 3A compared to the serotonin receptor 3AB (Dubin et al., 1999), the peak current (I_{5-HT}) elicited by 100 µM 5-HT was higher at the former (-28.3 ± 2.8 nA) compared to the latter receptor subtype (-8.3 ± 1.7 nA) (Table 3). To assess whether each congener inhibits 5-HT-evoked inward currents (I_{5-HT}), oocytes were co-treated (1 min) with 100 µM 5-HT and each separate congener (100 µM). The results indicated that none of the congeners inhibits the serotonin receptor 3A(Fig. 8B) or serotonin receptor 3AB receptor (Fig. 8C) subtype. One-way ANOVA analyses showed that 5-HT-evoked peak currents at the serotonin receptor 3A in the absence (-28.3 ± 2.8 nA) and the presence of (+)-catharanthine (-28.5 ± 1.7 nA) or (-)-18-MC (-29.0 ± 1.6 nA) are not significantly different ($p > 0.05$) (Table 3). Nevertheless, (-)-18-MC (2.2 ± 0.4 min), but not (+)-catharanthine (4.1 ± 0.4 min), was able to significantly reduce ($p < 0.001$) the deactivation recovery time elicited by 5-HT (5.0 ± 0.4 min) only at the serotonin receptor 3A (Table 3). During co-treatment, different conformational states of the receptor may coexist, including the active and desensitized states. (-)-18-MC neither activates nor inhibits serotonin receptor 3 currents, favoring the hypothesis that (-)-18-MC increases the desensitization process. Supporting this hypothesis, (-)-18-MC (0.39 ± 0.01), but not (+)-catharanthine (0.86 ± 0.09), significantly

reduced the normalized dopamine transporter charge (i.e., current amplitude area) with respect to that for 5-HT alone ($p < 0.001$).

To assess whether coronaridine congeners interact with serotonin receptors 3 and in the resting state, oocytes were preincubated (4 min) with 100 μM (+)-catharanthine or (-)-18-MC before 5-HT-induced activation. The results indicated that the congeners neither inhibit nor potentiate the serotonin receptor 3A (Fig. 8D) or serotonin receptor 3AB (Fig. 8E) subtype. However, both (-)-18-MC (2.9 ± 0.3 min) and (+)-catharanthine (2.5 ± 0.3 min) significantly reduced ($p < 0.001$) the deactivation recovery time only at the serotonin receptor 3A (Table 3). Our results suggest that both congeners interact with the serotonin receptor 3A in the resting state, facilitating the desensitization process by an allosteric mechanism that does not include I_{5-HT}. This is supported by a significant reduction of the normalized dopamine transporter charge values observed for both (-)-18-MC (0.43 ± 0.01) and (+)-catharanthine (0.43 ± 0.03) concerning that for 5-HT alone ($p < 0.001$) (Table 3). Since the deactivation recovery time and normalized dopamine transporter charge values for (-)-18-MC is practically the same as that in the pre-incubation protocol (Table 3), this congener also acts when the receptor is activated.
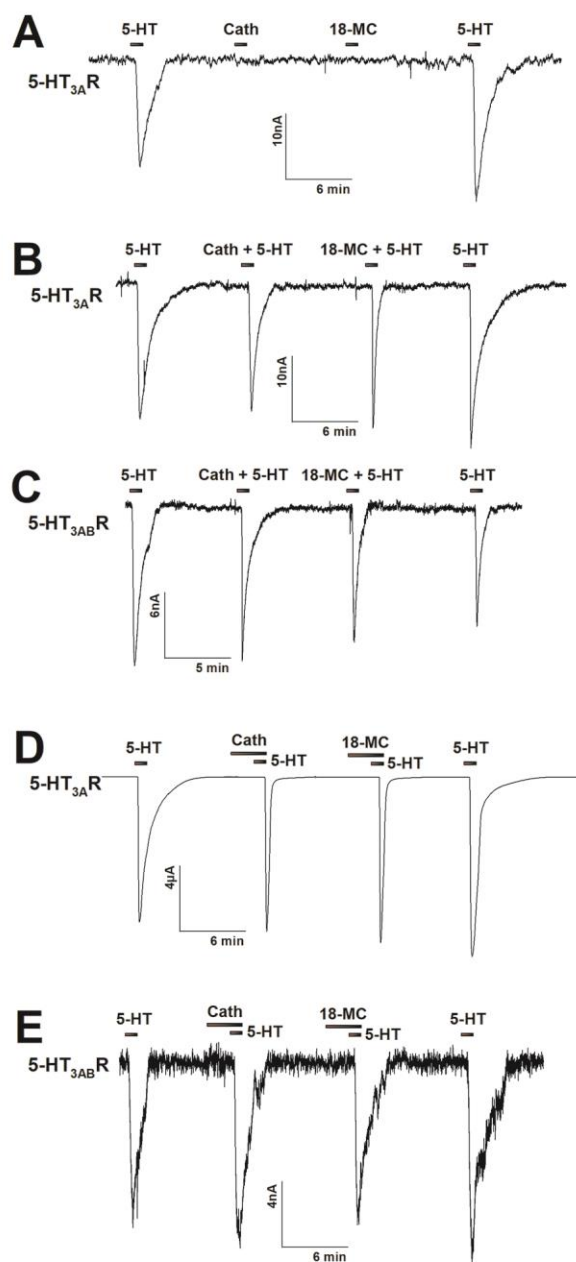
Figure 8. Effect of coronaridine congeners on serotonin receptor 3 function. (A) At a concentration of 100 μM, neither (+)-catharanthine (Cath) nor (-)-18-MC (18-MC) induced serotonin receptor 3A currents *per se*. Similar results were obtained with the serotonin receptor 3AB. (B,C) Co-treatment (1 min) with 100 μM 5-HT and each congener (100 μM) did not reduce 5-HT-evoked serotonin receptor 3A (B) or serotonin receptor 3AB (C) currents. (D,E) Pretreatment (4 min) with each congener (100 μM) neither

reduce nor potentiate 5-HT-evoked serotonin receptor 3A (D) or serotonin receptor 3AB (E) currents. 5-HT (100 µM) was used at the beginning and at the end of each experiment to show that functional serotonin receptors 3 had been expressed in oocytes. After each drug treatment, washing was performed for 15 min. The statistical analyses of the peak current, normalized dopamine transporter charge, and deactivation recovery time values were summarized in Table 3.

Table 3. The pharmacological activity of coronaridine congeners at human serotonin receptor 3A and serotonin receptor 3AB subtypes.

serotonin receptor 3 Subtype	Drug	Drug Treatment	Peak Current (nA)	Normalized Dopamine transporter Charge	Deactivation Recovery Time (min)
	5-HT	Alone	-28.3 ± 2.8	1.00 ± 0.05	5.0 ± 0.4
	(+)-Catharanthine	Co-treatment [a]	-28.5 ± 1.7	0.86 ± 0.09	4.1 ± 0.4
	(+)-Catharanthine	Pre-treatment [b]	-28.8 ± 1.2	0.43 ± 0.03 [c]	2.9 ± 0.3 [c]
serotonin receptor 3A	(-)-18-MC	Co-treatment [a]	-29.0 ± 1.6	0.39 ± 0.01 [c]	2.2 ± 0.4 [c]
	(-)-18-MC	Pre-treatment [b]	-28.4 ± 1.9	0.43 ± 0.01 [c]	2.5 ± 0.3 [c]
	5-HT	Alone	-8.3 ± 1.7	1.00 ± 0.17	1.9 ± 0.3
	(+)-Catharanthine	Co-treatment [a]	-8.2 ± 1.5	1.14 ± 0.05	2.1 ± 0.2
serotonin receptor 3AB	(+)-Catharanthine	Pre-treatment [b]	-8.8 ± 1.5	0.96 ± 0.06	2.2 ± 0.2
	(-)-18-MC	Co-treatment [a]	-9.5 ± 1.2	1.22 ± 0.02	1.9 ± 0.2
	(-)-18-MC	Pre-treatment [b]	-8.8 ± 2.1	1.07 ± 0.01	2.0 ± 0.2

4. Discussion

The main objective of the present study was to compare the antidepressant-like activity of (+)-catharanthine and (-)-18-MC between male and female mice, after acute and repeated treatments, and to determine plausible molecular mechanisms underlying the observed behavioral activities.

We determined the antidepressant-like activity of both (+)-catharanthine and (-)-18-MC after acute and repeated treatments using two different tests, FSTs and TSTs. We showed that both (+)-catharanthine and (-)-18-MC increase swimming time and decrease immobility time in the FST as well as decrease immobility time in the TST in a dose- and time-dependent, but sex-independent, manner. The results show that repeated treatments with 20 mg/kg of (+)-catharanthine or (-)-18-MC induced the same level of antidepressant-like activity compared to that for acute treatment using a dose of 40 mg/kg, indicating that prolonged treatments increase drug efficacy without inducing tolerance. The same trend was observed for the anti-addictive activity of 18-MC after prolonged treatment (Glick,Rossman, 1991).

The lack of sex-dependence in the antidepressant-like activity of both (+)-catharanthine and (-)-18-MC suggests that hormonal and neurochemical differences between male and female mice are not important for the antidepressant-like activity of coronaridine congeners. Of note, the activity of (-)-ibogaine against morphine-induced locomotor activity was found more robust in female than male rats, which was attributed to a higher bioavailability in the former animals (Pearl et al., 1997). A simple

explanation for this dichotomy is that the antidepressant-like effects of coronaridine congeners are mediated by different mechanisms than their anti-addictive activities.

Our results showing that coronaridine congeners induce antidepressant-like activity in an acute manner agree with previous studies with (-)-ibogaine and noribogaine (Rodrıguez,Urbanavicius, 2020). Nevertheless, there are interesting differences among congeners: (+)-catharanthine and (-)-18-MC showed antidepressant-like activity at 1 h that lasted until 24 h, whereas the activity of (-)-ibogaine and noribogaine did not last 24 h. Our acute TST results also showed an interesting distinction between (+)-catharanthine and (-)-18-MC, where the antidepressant-like activity of the former drug lasted for longer time (until 24 h) compared to the latter drug. The observed differences might reflect distinct metabolic, pharmacodynamics (see below), and/or pharmacokinetic properties among drugs. Although 18-MC (half-life ~10 min; (Glick,Maisonneuve, 1999), (-)-ibogaine (2-6 h; (Luz,Mash, 2021), and (+)-catharanthine (0.8-1.2 h; (Lin et al., 2015) are metabolized/cleared relatively quickly, longer behavioral activities were observed for each one of them (Glick,Maisonneuve, 1999; Glick,Rossman, 1991). The possibility that the observed prolonged activity is mediated by long-acting metabolites is supported by the observation that 18-hydroxycoronaridine (~100 min) and noribogaine (24-30 h; but see (Rodrıguez,Urbanavicius, 2020) last in plasma for longer time than the respective parental drug (Glick,Maisonneuve, 1999; Luz,Mash, 2021). However, we do not have evidence of active metabolites of (+)-catharanthine.

Our results suggest that the mechanisms at the origin of the antidepressant-like activity of (-)-18-MC and (+)-catharanthine slightly differed. In the FST, (-)-18-MC reduced immobility and increased swimming times without changing climbing behavior, whereas (+)-catharanthine reduced immobility time and increased both swimming time and climbing behavior. It has been previously reported the activity of antidepressants that reduce immobility and increases climbing behaviors without affecting swimming time is related to norepinephrine transporter inhibition (e.g., desipramine and reboxetine). However, bupropion,

which inhibits both the norepinephrine transporter and dopamine transporter, also increased climbing behaviors in rodents (Hayashi et al., 2011; Rénéric,Lucki, 1998). On the other hand, the activity of antidepressants that reduces immobility and increases swimming times without changing climbing behavior is related to serotonin transporter inhibition (e.g., fluoxetine) (Cryan,Markou, 2002; Sáenz et al., 2006). This consideration led us to hypothesize that the antidepressant-like activity of (-)-18-MC could involve serotonin transporter inhibition, whereas the antidepressant-like activity of (+)-catharanthine could also involve norepinephrine transporter inhibition.

To assess this hypothesis the binding and inhibitory properties of (+)-catharanthine and (-)-18-MC were respectively determined at the dopamine transporter, norepinephrine transporter, and the serotonin transporter, and compared to that of (-)-ibogaine, a well-characterized coronaridine congener (Bhat,Newman, 2019; Bulling,Schicker, 2012; Jacobs,Zhang, 2007). Our results indicated that (+)-catharanthine inhibits norepinephrine transporter with 3.9-fold higher potency than (-)-18-MC, whereas similar potency/affinity values for both congeners were determined at serotonin transporter. Based on previous results (Glick,Maisonneuve, 1999; Jacobs,Zhang, 2007) and our data, the following affinity/potency sequence is observed for serotonin transporter: noribogaine > (-)-ibogaine > (+)-catharanthine ~ (-)-18-MC. These results support the idea that the distinct affinity of both congeners toward serotonin and norepinephrine transporters could explain in part the distinct behavioral results,(-)-18-MC displaying a clear preferential action at the serotonin transporter.

To assess the role of serotonergic and norepinephrinergic neurotransmission in the antidepressant-like activity of (+)-catharanthine and (-)-18-MC, the levels of 5-HT and NE were selectively depleted by inhibiting 5-HT biosynthesis and inducing NE denervation, respectively. It is important to note that neither pCPA nor DSP-4 pretreatment modified the behavior of the animals. This finding is consistent with numerous data in the literature (Delaville et al., 2012; Page et al., 1999). Conversely, the results indicated

that 5-HT depletion decreases the acute antidepressant-like activity of both (+)-catharanthine and (-)-18-MC, whereas NE depletion only decreases (+)-catharanthine's activity. This profile of responses of both congeners corresponds to their *in vitro* potencies for the respective blocking of the serotonin transporter and norepinephrine transporter.

In general, congeners have higher binding affinity compared to their inhibitory potencies. The exception is (-)-18-MC, which has higher inhibitory potency compared to its binding affinity for the norepinephrine transporter. The discrepancy between binding and uptake inhibition can be rationalized by taking into account that the ionic conditions in the binding assays favor the outward-facing state of the transporter. In contrast, during uptake inhibition, the transporters sequentially adopt several conformations as they move through the transport cycle. For atypical inhibitors, this can *per se* result in very large discrepancies in affinity estimates (Bhat et al., 2017). The transport cycle of the norepinephrine transporter is characterized by a very slow return step (Bhat,Guthrie, 2021). A compound that binds preferentially to the inward-facing state of the norepinephrine transporter is predicted to have a higher potency for inhibition of uptake than for binding. Thus, based on our findings, (-)-18-MC is likely to be highly selective for the inward-facing state of norepinephrine transporter.

Additional experiments with serotonin receptor 3A and serotonin receptor 3AB subtypes showed that although (+)-catharanthine and (-)-18-MC does not activate, potentiate, or inhibit serotonin receptor 3 currents, they decrease both deactivation recovery time and normalized dopamine transporter charge at the serotonin receptor 3A subtype. This type of response suggests that the two compounds increase the desensitization process by an allosteric mechanism at this receptor subtype. Since the deactivation recovery time and normalized dopamine transporter charge values for (-)-18-MC were practically the same as that in the preincubation protocol, it is possible that this congener also acts when the receptor is

activated. Despite the interest in serotonin receptor 3 antagonism in the mechanism of action of antidepressant drugs (Okada et al., 2019; Riga et al., 2020; Riga et al., 2016), our pharmacological study indicates that the antidepressant-like activity of both congeners is unlikely related to serotonin receptor 3.

In summary, our behavioral study showed that (+)-catharanthine and (-)-18-MC induce antidepressant-like activity in a dose- and time-dependent, and sex-independent, manner. The antidepressant-like activity of both congeners involves serotonin transporter inhibition, and consequently the recruiting of serotonergic pathways, without activating or inhibiting serotonin receptors 3, whereas (+)-catharanthine's activity is also mediated by norepinephrinergic pathways through norepinephrine transporter inhibition.

Acknowledgments

Author contributions

HRA developed the concept, HRA and AC wrote the manuscript. AC conducted the behavioral studies. AE-K performed the transporter studies. SE performed the electrophysiological studies. All co-authors wrote the methods, performed data analyses, and contributed to critical comments on the manuscript and discussion.

References

Amidfar, M.,Kim, Y. K., 2018. Recent Developments on Future Antidepressant-related Serotonin Receptors. Curr Pharm Des 24(22), 2541-2548 https://doi.org/10.2174/1381612824666180803111240.

Arias, H. R., Do Rego, J. L., Do Rego, J. C., Chen, Z., Anouar, Y., Scholze, P., Gonzales, E. B., Huang, R.,Chagraoui, A., 2020a. Coronaridine congeners potentiate GABA(A) receptors and induce sedative activity in mice in a benzodiazepine-insensitive manner. Prog Neuropsychopharmacol Biol Psychiatry 101, 109930 https://doi.org/10.1016/j.pnpbp.2020.109930.

Arias, H. R., Jin, X., Feuerbach, D.,Drenan, R. M., 2017. Selectivity of coronaridine congeners at nicotinic acetylcholine receptors and inhibitory activity on mouse medial habenula. Int J Biochem Cell Biol 92, 202-209 https://doi.org/10.1016/j.biocel.2017.10.006.

Arias, H. R., Tae, H.-S., Micheli, L., Yousuf, A., Ghelardini, C., Adams, D. J.,Di Cesare Mannelli, L., 2020b. Coronaridine congeners decrease neuropathic pain in mice and inhibit α9α10 nicotinic acetylcholine receptors and CaV2.2 channels. Neuropharmacology 175, 108194 https://doi.org/https://doi.org/10.1016/j.neuropharm.2020.108194.

Arias, H. R., Targowska-Duda, K. M., Feuerbach, D.,Jozwiak, K., 2015. Coronaridine congeners inhibit human alpha3beta4 nicotinic acetylcholine receptors by interacting with luminal and non-luminal sites. Int J Biochem Cell Biol 65, 81-90 https://doi.org/10.1016/j.biocel.2015.05.015.

Bhat, S., Guthrie, D. A., Kasture, A., El-Kasaby, A., Cao, J., Bonifazi, A., Ku, T., Giancola, J. B., Hummel, T., Freissmuth, M.,Newman, A. H., 2021. Tropane-Based Ibogaine Analog Rescues Folding-Deficient Serotonin and Dopamine Transporters. ACS Pharmacol Transl Sci 4(2), 503-516 https://doi.org/10.1021/acsptsci.0c00102.

Bhat, S., Hasenhuetl, P. S., Kasture, A., El-Kasaby, A., Baumann, M. H., Blough, B. E., Sucic, S., Sandtner, W.,Freissmuth, M., 2017. Conformational state interactions provide clues to the pharmacochaperone potential of serotonin transporter partial substrates. J Biol Chem 292(40), 16773-16786 https://doi.org/10.1074/jbc.M117.794081.

Bhat, S., Newman, A. H.,Freissmuth, M., 2019. How to rescue misfolded SERT, DAT and NET: targeting conformational intermediates with atypical inhibitors and partial releasers. Biochem Soc Trans 47(3), 861-874 https://doi.org/10.1042/bst20180512.

Bulling, S., Schicker, K., Zhang, Y. W., Steinkellner, T., Stockner, T., Gruber, C. W., Boehm, S., Freissmuth, M., Rudnick, G., Sitte, H. H.,Sandtner, W., 2012. The mechanistic basis for noncompetitive ibogaine inhibition of serotonin and dopamine transporters. J Biol Chem 287(22), 18524-18534 https://doi.org/10.1074/jbc.M112.343681.

Carnicella, S., He, D. Y., Yowell, Q. V., Glick, S. D.,Ron, D., 2010. Noribogaine, but not 18-MC, exhibits similar actions as ibogaine on GDNF expression and ethanol self-administration. Addict Biol 15(4), 424-433 https://doi.org/10.1111/j.1369-1600.2010.00251.x.

Cryan, J. F., Kelly, P. H., Neijt, H. C., Sansig, G., Flor, P. J.,van Der Putten, H., 2003. Antidepressant and anxiolytic-like effects in mice lacking the group III metabotropic glutamate receptor mGluR7. Eur J Neurosci 17(11), 2409-2417 https://doi.org/10.1046/j.1460-9568.2003.02667.x.

Cryan, J. F., Markou, A.,Lucki, I., 2002. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 23(5), 238-245 https://doi.org/10.1016/s0165-6147(02)02017-5.

Cryan, J. F., Mombereau, C.,Vassout, A., 2005a. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. Neuroscience & Biobehavioral Reviews 29(4-5), 571-625 https://doi.org/10.1016/j.neubiorev.2005.03.009

Delaville, C., Chetrit, J., Abdallah, K., Morin, S., Cardoit, L., De Deurwaerdère, P.,Benazzouz, A., 2012. Emerging dysfunctions consequent to combined monoaminergic depletions in Parkinsonism. Neurobiol Dis 45(2), 763-773 https://doi.org/10.1016/j.nbd.2011.10.023.

Dubin, A. E., Huvar, R., D'Andrea, M. R., Pyati, J., Zhu, J. Y., Joy, K. C., Wilson, S. J., Galindo, J. E., Glass, C. A., Luo, L., Jackson, M. R., Lovenberg, T. W.,Erlander, M. G., 1999. The pharmacological and functional characteristics of the serotonin 5-HT(3A) receptor are specifically modified by a 5-HT(3B) receptor subunit. J Biol Chem 274(43), 30799-30810 https://doi.org/10.1074/jbc.274.43.30799.

Eckeli, A. L., Dach, F.,Rodrigues, A. L. S., 2000. Acute treatments with GMP produce antidepressant-like effects in mice. Neuroreport 11(9), 1839-1843   https://doi.org/10.1097/00001756-200006260-00008

Glick, S. D., Maisonneuve, I. M., Hough, L. B., Kuehne, M. E.,Bandarage, U. K., 1999. (±)‑18‑Methoxycoronaridine: a novel iboga alkaloid congener having potential anti‑addictive efficacy. CNS Drug Reviews 5(1), 27-42 https://doi.org/10.1111/j.1527-3458.1999.tb00084.x

Glick, S. D., Rossman, K., Steindorf, S., Maisonneuve, I. M.,Carlson, J. N., 1991. Effects and aftereffects of ibogaine on morphine self-administration in rats. Eur J Pharmacol 195(3), 341-345 https://doi.org/10.1016/0014-2999(91)90474-5.

Glick, S. D., Sell, E. M., McCallum, S. E.,Maisonneuve, I. M., 2011. Brain regions mediating alpha3beta4 nicotinic antagonist effects of 18-MC on nicotine self-administration. Eur J Pharmacol 669(1-3), 71-75 https://doi.org/10.1016/j.ejphar.2011.08.001.

Hayashi, E., Shimamura, M., Kuratani, K., Kinoshita, M.,Hara, H., 2011. Automated experimental system capturing three behavioral components during murine forced swim test. Life Sciences 88(9), 411-417 https://doi.org/https://doi.org/10.1016/j.lfs.2010.12.016.

Jacobs, M. T., Zhang, Y. W., Campbell, S. D.,Rudnick, G., 2007. Ibogaine, a noncompetitive inhibitor of serotonin transport, acts by stabilizing the cytoplasm-facing state of the transporter. J Biol Chem 282(40), 29441-29447 https://doi.org/10.1074/jbc.M704456200.

Lee, S., Seol, H. S., Eom, S., Lee, J., Kim, C., Park, J. H., Kim, T. H.,Lee, J. H., 2022. Hydroxy Pentacyclic Triterpene Acid, Kaempferol, Inhibits the Human 5-Hydroxytryptamine Type 3A Receptor Activity. Int J Mol Sci 23(1) https://doi.org/10.3390/ijms23010544.

Lin, C., Cai, J., Yang, X., Hu, L.,Lin, G., 2015. Liquid chromatography mass spectrometry simultaneous determination of vindoline and catharanthine in rat plasma and its application to a pharmacokinetic study. Biomed Chromatogr 29(1), 97-102 https://doi.org/10.1002/bmc.3244.

Lino-de-Oliveira, C., De Lima, T. C.,de Pádua Carobrez, A., 2005. Structure of the rat behaviour in the forced swimming test. Behav Brain Res 158(2), 243-250 https://doi.org/10.1016/j.bbr.2004.09.004.

Luz, M.,Mash, D. C., 2021. Evaluating the toxicity and therapeutic potential of ibogaine in the treatment of chronic opioid abuse. Expert Opin Drug Metab Toxicol 17(9), 1019-1022 https://doi.org/10.1080/17425255.2021.1944099.

Maisonneuve,Glick, 2003. Anti-addictive actions of an iboga alkaloid congener: a novel mechanism for a novel treatment. Pharmacol Biochem Behav 75(3), 607-618 https://doi.org/10.1016/s0091-3057(03)00119-9.

Mayorga, A. J.,Lucki, I., 2001. Limitations on the use of the C57BL/6 mouse in the tail suspension test. Psychopharmacology 155(1), 110-112 https://doi.org/10.1007/s002130100687

Mineur, Y. S., Cahuzac, E. L., Mose, T. N., Bentham, M. P., Plantenga, M. E., Thompson, D. C.,Picciotto, M. R., 2018. Interaction between noradrenergic and cholinergic signaling in amygdala regulates anxiety- and depression-related behaviors in mice. Neuropsychopharmacology 43(10), 2118-2125 https://doi.org/10.1038/s41386-018-0024-x.

Okada, M., Okubo, R.,Fukuyama, K., 2019. Vortioxetine Subchronically Activates Serotonergic Transmission via Desensitization of Serotonin 5-HT(1A) Receptor with 5-HT(3) Receptor Inhibition in Rats. Int J Mol Sci 20(24) https://doi.org/10.3390/ijms20246235.

Page, M. E., Detke, M. J., Dalvi, A., Kirby, L. G.,Lucki, I., 1999. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. Psychopharmacology (Berl) 147(2), 162-167 https://doi.org/10.1007/s002130051156.

Papke, R. L.,Porter Papke, J. K., 2002. Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis. Br J Pharmacol 137(1), 49-61 https://doi.org/10.1038/sj.bjp.0704833.

Pearl, S. M., Hough, L. B., Boyd, D. L.,Glick, S. D., 1997. Sex differences in ibogaine antagonism of morphine-induced locomotor activity and in ibogaine brain levels and metabolism. Pharmacol Biochem Behav 57(4), 809-815 https://doi.org/10.1016/s0091-3057(96)00383-8.

Perona, M. T., Waters, S., Hall, F. S., Sora, I., Lesch, K. P., Murphy, D. L., Caron, M.,Uhl, G. R., 2008. Animal models of depression in dopamine, serotonin, and norepinephrine transporter

knockout mice: prominent effects of dopamine transporter deletions. Behav Pharmacol 19(5-6), 566-574 https://doi.org/10.1097/FBP.0b013e32830cd80f.

Rénéric, J. P.,Lucki, I., 1998. Antidepressant behavioral effects by dual inhibition of monoamine reuptake in the rat forced swimming test. Psychopharmacology 136(2), 190-197 https://doi.org/10.1007/s002130050555.

Riga, M. S., Sanchez, C., Celada, P.,Artigas, F., 2020. Sub-chronic vortioxetine (but not escitalopram) normalizes brain rhythm alterations and memory deficits induced by serotonin depletion in rats. Neuropharmacology 178, 108238 https://doi.org/10.1016/j.neuropharm.2020.108238.

Riga, M. S., Sánchez, C., Celada, P.,Artigas, F., 2016. Involvement of 5-HT3 receptors in the action of vortioxetine in rat brain: Focus on glutamatergic and GABAergic neurotransmission. Neuropharmacology 108, 73-81 https://doi.org/10.1016/j.neuropharm.2016.04.023.

Rodrıguez, P., Urbanavicius, J., Prieto, J. P., Fabius, S., Reyes, A. L., Havel, V., Sames, D., Scorza, C.,Carrera, I., 2020. A Single Administration of the Atypical Psychedelic Ibogaine or Its Metabolite Noribogaine Induces an Antidepressant-Like Effect in Rats. ACS Chem Neurosci 11(11), 1661-1672 https://doi.org/10.1021/acschemneuro.0c00152.

Ross, S. B.,Stenfors, C., 2015. DSP4, a selective neurotoxin for the locus coeruleus noradrenergic system. A review of its mode of action. Neurotox Res 27(1), 15-30 https://doi.org/10.1007/s12640-014-9482-z.

Sáenz, J. C. B., Villagra, O. R.,Trías, J. F., 2006. Factor analysis of forced swimming test, sucrose preference test and open field test on enriched, social and isolated reared rats. Behavioural brain research 169(1), 57-65 https://doi.org/10.1016/j.bbr.2005.12.001

Solt, K., Ruesch, D., Forman, S. A., Davies, P. A.,Raines, D. E., 2007. Differential effects of serotonin and dopamine on human 5-HT3A receptor kinetics: interpretation within an allosteric kinetic model. J Neurosci 27(48), 13151-13160 https://doi.org/10.1523/jneurosci.3772-07.2007.

Steru, L., Chermat, R., Thierry, B.,Simon, P., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85(3), 367-370 https://doi.org/10.1007/bf00428203

Zomkowski, A. D. E., Rosa, A. O., Lin, J., Santos, A. R., Calixto, J. B.,Rodrigues, A. L. S., 2004. Evidence for serotonin receptor subtypes involvement in agmatine antidepressant like-effect in the mouse forced swimming test. Brain research 1023(2), 253-263 https://doi.org/10.1016/j.brainres.2004.07.041

Response to the comments of Reviewer #2

Thank you very much for the interest you have shown in reviewing our manuscript and for your appreciated comments:

1. In figure 4 (and also in figure 2) there is a large difference in climbing scores between catharantine and 18MC groups? Authors should explain what the reason for this difference is.

2. To this reviewer it is not clear enough the association between the drugs' affinities for serotonin or noradrenaline transporters and their effects in vivo. Authors should give a clearer panorama.

Response to the comments 1 and 2 :

We understand the comments of the reviewer. We believe that the lack of clarity was related to the organization of some paragraphs in the discussion. We have thus reorganized the paragraphs in the discussion to maintain the flow and the logic. This part (page 32,33 and 34) now sequentially address 1) the distinct behavioral response of the two congeners, 2) their distinct affinity toward the serotonin and the norepinephrine transporters, and 3) the distinct contribution of noradrenergic and serotonergic pathways in their effect.

3. Please check the spelling of swimming in all figures. In the X axis it is misspelled.

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We have corrected the word swimming on all figures

Instructions for style revision:

Special attention should be given to the following items:

1. Carefully check for spelling and for grammar mistakes

Response to the comments 1 :

We checked for spelling and grammatical errors.

2. Abbreviations

Authors should not use laboratory jargon or shorthand, both of which are a hindrance to the reader.

Explain abbreviations when they are used for the first time in abstract and text

Authors must use as few abbreviations as possible.

Response to the comments 2 :

We have used as few abbreviations as possible and descrived the first time were introduced.

3. Nomenclature

Proper nomenclature for drugs, peptides and receptors must be used.

Peptide nomenclature is as in: J. Biol. Chem. 260 (1983) 14-42.

Receptor, ion channel and transporter nomenclature is according to the IUPHAR receptor database (http://www.iuphar-db.org/iuphar-rd/)

For receptors avoid the abbreviation 'R'. With receptor subtypes mention the full receptor name throughout the text: e.g. adenosine A1 receptor, melanocortin MC3 receptor, endothelin ET1 receptor.

Response to the comments 3 :

we have fixed the names of the receptors according to the suggested nomenclature.

4. Spacing

The manuscript, including title page, references, legend to tables and figures, should be typed double-spaced (at least 6mm between lines).

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6. Tables

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Avoid vertical lines

Response to the comments 6

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7. Figures

Submit high- resolution files of each figure

Response to the comments 7

The figures have been edited in tif format (high resolution)

credit author statement

Author contributions :

HRA developed the concept, HRA and AC wrote the manuscript. AC conducted the behavioral studies. AE-K performed the transporter studies. SE performed the electrophysiological studies. All co-authors wrote the methods, performed data analyses, and contributed to critical comments on the manuscript and discussion.

All authors have made substantial contributions to the preparation of the manuscript. Furthermore, they have not only contributed to the writing of the final manuscript but have given their permission for submission and publication in European Journal of Pharmacology.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

A Chagraoui

Rouen, November 29, 2022

Ms. Ref. EJP-62740

Title: (+)-Catharanthine and (-)-18-methoxycoronaridine induce antidepressant-like

activity in mice by differently recruiting serotonergic and norepinephrinergic

neurotransmission

Dear Pr. F.A. Redegeld , Editor in chief,

We are grateful for your consideration of this manuscript, and we also very much appreciate your suggestions, which have been very helpful in improving the manuscript. We also thank the reviewer for their careful reading of our text and suggestions. We have revised the manuscript accordingly and we hope that it can now be acceptable for EJP.
Please, find the answers to the reviewer's criticisms below.

We remain at your entire disposal for any clarification or documentary proof that you might

require.

Yours sincerely,

Abdeslam Chagraoui , PhD., PharmD.

Please find below the editing comments and responses to reviewers' comments :

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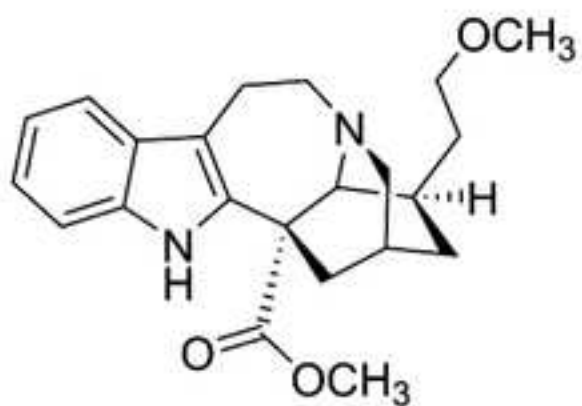
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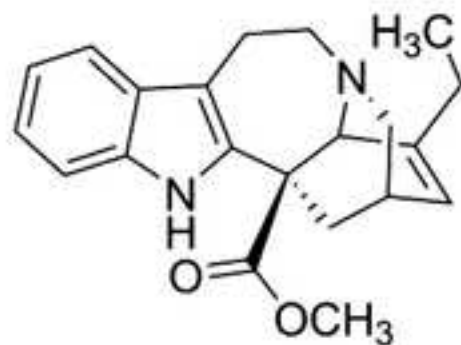*The tables have been fixed in the style of the journal and typed double-spaced*

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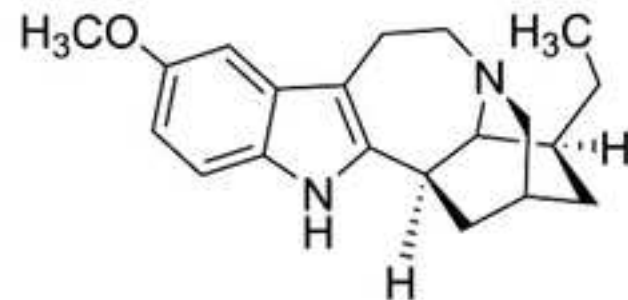Submit high- resolution files of each figure

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(-)-18-MC   (+)-Catharanthine   (-)-Ibogaine

Figure 2

Figure 3

Figure 4

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Figure 5

Figure 6

Figure 7

Figure 8

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A 5-HT₃ₐR

B 5-HT₃ₐR

C 5-HT₃ₐᵦR

D 5-HT₃ₐR

E 5-HT₃ₐᵦR