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Gamma-decanolactone: Preliminary evaluation as potential antiparkinsonian drug

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ABSTRACT

Treatment of Parkinson's disease (PD) includes the use of monoamine oxidase-B (MAO-B) inhibitor drugs. In this work we have evaluated the possible gamma-decanolactone (GD) effect *in vitro* to inhibit the A and B isoforms of human monoamine oxidase (hMAO) enzyme and their citotoxicity in human hepatoma cell line (HepG2). Also, binding studies to A_1, A_{2A} A_{2B} and A_3 adenosine receptors were performed.

A docking study of gamma-decanolactone has been carried out with the molecular targets of MAO-A and MAO-B isoforms.

The physicochemical properties and ability to cross physiological barriers, as the blood brain barrier (BBB), was elucidated by computational studies.

The *in vivo* assays, the rota-rod test, body temperature assessment and open field test were performed in reserpinized mice (1.5 mg/kg, i.p.; 18:00 before) to evaluate the effect of gamma-decanolactone (300 mg/kg), alone or associated with Levodopa plus Benserazide (LD + BZ, 100:25 mg/kg, i.p.).

Gamma-decanolactone inhibited preferentially the MAO-B in a reversible manner, with an inhibitory concentration of 50% (IC_{50}) 55.95 ± 9.06 μM. It was shown to be a safe drug since only at the highest concentration decreased the viability of HepG2 cells. It also does not bind to adenosine receptors investigated in this study.

The molecular docking study show that the gamma-decanolactone ligand adopts a relatively compact conformation in the active site of hMAO-B, while we note an extended conformation of gamma-decanolactone ligand in the hMAO-A isoform.

The physicochemical properties obtained, and the theoretical models utilized for the evaluation of ability to cross the BBB, predict a good gamma-decanolactone bioavailability and access to the central nervous system (CNS).

In the *in vivo* studies, gamma-decanolactone partially reversed the ataxia of the reserpinized mice at 01:00 h and 01:30 h post-administration. Concomitant treatment of gamma-decanolactone with LD + BZ, at 01:30 h showed a potentiation of the reversibility of ataxia and facilitated the reversal of hypothermia caused by reserpine for all measured times ($P < 0.01$ *vs* vehicle), except at 24:00 h, but not reversed the hypokinesia in the open field test.

In summary, the results herein obtained and in conjunction with previous studies, suggest that gamma-decanolactone could be a drug with potential utility as antiparkinsonian drug.

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1. Introduction

Parkinson's disease (PD) is considered a neurodegenerative, chronic, and progressive disease of the central nervous system (CNS) that affects more than 1% of the worldwide population above the age of 65 (Angelopoulou et al., 2020). The main clinical features of PD involve motor symptoms such as resting tremor, rigidity, bradykinesia, and postural instability. The histopathologic hallmarks of PD are a progressive decrease in the dopamine (DA) production in the nigrostriatal pathway and the accumulation of misfolded alpha-synuclein protein (Brettschneider et al., 2015; Sasikumar and Strafella, 2020; Van Den Eeden et al., 2003).

It has been postulated that the Parkinson's disease and other neurodegenerative diseases, such as Alzheimer's disease (AD), and some neurological diseases, as epilepsy, can have etiologies that are intrinsically related. These diseases share some similar pathological mechanisms, such as neuronal loss, increased excitotoxicity, higher oxidative stress and pro-inflammatory activity and more production of apoptotic cytokines, among others (Angelopoulou et al., 2020; Brettschneider et al., 2015; Matos et al., 2013).

Clinically active drugs currently on the pharmaceutical market for the treatment of Parkinson's disease include the dopamine precursor levodopa (LD), the dopadescarboxilase inhibitors, the dopaminergic receptor agonists, the catechol-O-methyltransferase (COMT) inhibitors and monoamine oxidase B (MAO-B) inhibitors and, in minor degree, the anticholinergic drugs (Homayoun, 2018; Huang et al., 2019; Young et al., 1997).

However, some anti-Parkinson drugs could produce serious side effects. The dopamine precursor levodopa was associated with increased oxidative stress (Fenton reaction), the ergotic alkaloid dopaminergic agonists, as bromocriptine, with fibrotic reactions and the non-ergotic derivatives with sleep tendence (Yeung and Cavanna, 2014). In the MAO-B inhibitors group, selegiline and rasagiline act as irreversible inhibitors. Also, selegiline is associated with the production of neurotoxic amphetamine metabolites (Delogu et al., 2017; Matos et al., 2013; Riederer and Müller, 2018). Cognitive impairment, urinary retention, constipation, etc, are well-known side effects of anticholinergic drugs which represent a very serious problem in elderly patients (López-Álvarez et al., 2019).

As consequence of above-mentioned problems, is important the development of more effective and safety drugs. Safinamide is a new selective and reversible monoamine oxidase-B inhibitor (MAOI-B) causing an increase in extracellular levels of dopamine in the striated body (Blair and Dhillon, 2017).

Regarding the multi-target strategies for Parkinson's disease treatment, use of MAO-B inhibitors with adenosine A_{2A} receptor antagonist activity is also being study (Jaiteh et al., 2018; Kuder et al., 2020).

It has been previously described that gamma-decanolactone (GD), Fig. 1, is effective on blocking acute or chronic seizures, induced by different chemical agents in mice (Coelho et al., 1997; De Oliveira et al., 2008; Pflüger et al., 2018a, 2018b).

The gamma-decanolactone was able to dose-dependently inhibit the specific binding of L-[^{3}H]-glutamate, it has a possible implication in the modulation of the glutamic acid decarboxylase (GAD) enzyme, and it acts on K^+ channels, leading to a reduction in excitotoxicity. Also, it decreases the expression of inducible nitric oxide synthase (iNOS) and tumor necrosis factor alpha (TNF-α) in microglial cells and consequently inhibits the formation of reactive oxygen species (ROS) (Pereira et al., 1997; Pflüger et al., 2016; 2018b). Also, it blocks apoptosis markers such as p38 phosphorylation and the activation of cleaved caspase-9, decreasing deoxyribonucleic acid (DNA) damage, demonstrating that its neuroprotective therapeutic potential comes from inhibiting neuroinflammation.

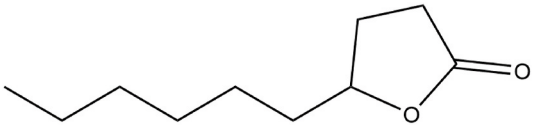

Fig. 1. Structure of gamma-decanolactone (GD).

Considering the mechanisms of action in which gamma-decanolactone has already proved efficient and the similarity of these mechanisms with the pathophysiology of neurodegenerative diseases such as Parkinson's disease, we think that gamma- decanolactone may have a neuroprotective effect and prevent or minimize motor signs related to Parkinson's disease.

To address this question, in the present work, we have evaluated *in vitro* the potential of the gamma-decanolactone for the MAO-A and MAO-B inhibition, the binding to adenosine receptors, and cytotoxicity. Also, docking studies to the two MAO isoforms were performed.

The theoretical physicochemical properties and ability to cross physiological barriers was evaluated by mean of computational studies (*in silico*).

Finally, *in vivo* gamma-decanolactone evaluation, related to behavioral parameters (rotarod test, body temperature assessment and open field test) on reserpinized mouse model, has been performed.

2. Material and methods

2.1. In vitro assays

2.1.1. Determination of human MAO (hMAO) isoform activity

The effects of the gamma-decanolactone on the enzymatic activity of hMAO isoforms were evaluated by a fluorimetric method. Briefly, 0.1 ml of sodium phosphate buffer (0.05 M, pH 7.4) containing different concentrations of gamma-decanolactone or reference inhibitors and adequate amounts of recombinant hMAO-A or hMAO-B required and adjusted to obtain under our experimental conditions the same reaction velocity [165 pmol of *p*-tyramine per min (hMAO-A: 1.1 μg of protein; specific activity: 150 nmol of *p*-tyramine oxidized to *p*-hydroxyphenylacetaldehyde/min/mg of protein; hMAOB: 7.5 μg of protein; specific activity: 22 nmol of *p*-tyramine transformed/min/mg of protein)] were placed in a dark fluorimeter chamber and incubated for 10 min at 37 °C. The reaction was started by adding (final concentrations) 200 μM Amplex Red reagent, 1 U/ml horseradish peroxidase and 1 mM *p*-tyramine. The production of hydrogen peroxide (H_2O_2) and, consequently, of resorufin was quantified at 37 °C in a multidetection microplate fluorescence reader (Fluo-Star Optima, BMG LABTECH, Offenburg, Germany) based on the fluorescence generated (excitation, 545 nm, emission, 590 nm) over a 15 min period, in which the fluorescence increased linearly. Control experiments were carried out simultaneously by replacing the tested drugs with appropriate dilutions of the vehicles. In addition, the possible capacity of the above tested drugs for modifying the fluorescence generated in the reaction mixture due to non-enzymatic inhibition (e.g., for directly reacting with Amplex Red reagent) was determined by adding these drugs to solutions containing only the Amplex Red reagent in a sodium phosphate buffer. The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of the background activity, which was determined from wells containing all components except the hMAO isoforms, which were replaced by a sodium phosphate buffer solution (Yáñez et al., 2006).

2.1.2. Reversibility

A dilution method was used to evaluate whether gamma-decanolactone is a reversible or irreversible hMAO-B inhibitor (Copeland, 2005; Gerlach et al., 1992). A 100X concentration of the enzyme used in the above described experiments was incubated with a concentration of inhibitor equivalent to 10-fold the IC_{50} value. After 30 min, the mixture was diluted 100-fold into a reaction buffer containing Amplex Red reagent, horseradish peroxidase and *p*-tyramine and the

reaction was monitored for 15 min. Reversible inhibitors show linear progress with a slope equal to ≈91% of the slope of the control, whereas irreversible inhibition reaches only ≈9% of this slope. A control test was carried out by pre-incubating and diluting the enzyme in the absence of inhibitor.

2.1.3. Determination of gamma-decanolactone binding at human A_1, A_{2A}, A_{2B} and A_3 adenosine receptors

2.1.3.1. Competition binding in human A_1 adenosine receptors. Adenosine A_1 receptor competition binding experiments were carried out in a multiscreen GF/C 96-well plate (Millipore, Madrid, Spain) pretreated with binding buffer (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (Hepes) 20 mM, sodium chloride (NaCl) 100 mM, magnesium dichloride ($MgCl_2$) 10 mM, 2 U/ml adenosine deaminase, pH=7.4). In each well were incubated 5 μg of membranes from Euroscreen Chinese hamster ovary-A1 cell line (CHO-A1) and prepared in our laboratory (Lot: A002/13-04-2011, protein concentration=5864 μg/ml), 1 nM [^{3}H]- 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (137 Ci/mmol, 1 mCi/ml, Perkin Elmer NET974001MC) and gamma-decanolactone or standard. Non-specific binding was determined in the presence of (R)-N6-(1-methyl-2-phenylethyl) adenosine (R-PIA) 10 μM (Sigma P4532). The reaction mixture (Vt: 200 μl/well) was incubated at 25 °C for 60 min, after was filtered and washed four times with 250 μl wash buffer (Hepes 20 mM, NaCl 100 mM, $MgCl_2$ 10 mM pH=7.4), before measuring in a microplate beta scintillation counter (MicrobetaTrilux, PerkinElmer, Madrid, Spain).

2.1.3.2. Competition binding in human A_{2A} adenosine receptors. Adenosine A_{2A} receptor competition binding experiments were carried out in a multiscreen GF/C 96-well plate (Millipore, Madrid, Spain) pretreated with binding buffer (hydroxymethyl-amino-methane hydrochloride (Tris-HCl) 50 mM, ethylenediamine tetraacetic acid (EDTA) 1 mM, $MgCl_2$ 10 mM, 2 U/ml adenosine deaminase, pH=7.4). In each well were incubated 5 μg of membranes from human cervical adenocarcinoma epithelial- A_{2A} cell line (Hela-A_{2A}) and prepared in our laboratory (Lot: A002/17-04-2018, protein concentration=2058 μg/ml), 3 nM [^{3}H]- 4-(2-((7-amino-2-(furan-2-il)-[1,2,4]triazolo[1,5-a]-[1,3,5]triazin-5-il) amino)etil)fenol (ZM241385) (50 Ci/mmol, 1 mCi/ml, ARC-ITISA 0884) and gamma-decanolactone or standard. Non-specific binding was determined in the presence of 5′-(N-ethylcarboxamido) adenosine (NECA) 50 μM (Sigma E2387). The reaction mixture (Vt: 200 μl/well) was incubated at 25 °C for 30 min, after was filtered and washed four times with 250 μl wash buffer (Tris-HCl 50 mM, EDTA 1 mM, $MgCl_2$ 10 mM, pH=7.4), before measuring in a microplate beta scintillation counter (MicrobetaTrilux, PerkinElmer, Madrid, Spain).

2.1.3.3. Competition binding in human A_{2B} adenosine receptors. Adenosine A_{2B} receptor competition binding experiments were carried out in a multiscreen GF/C 96-well plate. In each well were incubated 25 μg of membranes from Euroscreen human embryonic kidney- A_{2B} cell line (HEK-A_{2B}) and prepared in our laboratory (Lot: A008/30-04- 2019, protein concentration=3916.02 μg/ml), 25 nM [^{3}H]-DPCPX (137 Ci/mmol, 1 mCi/ml, Perkin Elmer NET974001MC) and gamma-decanolactone or standard. Non-specific binding was determined in the presence of NECA 1000 μM (Sigma E2397). The reaction mixture (Vt: 250 μl/well) was incubated at 25 °C for 30 min, 200 μl were transferred to GF/C 96-well plate (Millipore, Madrid, Spain) pretreated with binding buffer (Tris-HCl 50 mM, EDTA 1 mM, $MgCl_2$ 5 mM, Bacitracin 100 μg/μl, adenosine deaminase 2 U/ml, pH=6.5), after was filtered and washed four times with 250 μl wash buffer (Tris-HCl 50 mM, EDTA 1 mM, $MgCl_2$ 5 mM, pH=6.5), before measuring in a microplate beta scintillation counter (MicrobetaTrilux, PerkinElmer, Madrid, Spain).

2.1.3.4. Competition binding in human A_3 adenosine receptors. Adenosine A_3 receptor competition binding experiments were carried out in a multiscreen GF/B 96-well plate (Millipore, Madrid, Spain) pretreated with binding buffer (Tris-HCl 50 mM, EDTA 1 mM, $MgCl_2$ 5 mM, 2 U/ml 2 adenosine deaminase, pH=7.4). In each well were incubated 30 μg of membranes from Hela-A_3 cell line and prepared in our laboratory (Lot: A005/05-07-2019, protein concentration=3925 μg/ml), 10 nM [^{3}H]-NECA (26.3 Ci/mmol, 1 mCi/ml, Perkin Elmer NET811250UC) with compound studied and standard. Non-specific binding was determined in the presence of R-PIA 100 μM (Sigma P4532). The reaction mixture (Vt: 200 μl/well) was incubated at 25 °C for 180 min, after was filtered and washed six times with 250 μl wash buffer (Tris-HCl 50 mM pH=7.4), before measuring in a microplate beta scintillation counter (MicrobetaTrilux, PerkinElmer, Madrid, Spain).

2.1.4. Cytotoxicity

Cells of the human hepatoma cell line (HepG2) from ATCC® (generously facilited/supplied by Research Group Biofarma CiMUS, USC), which is used widely in experimental studies for hepatotoxicity evaluation were used in the experiments. Cells were cultured in Eagle's Minimum Essential Medium (EMEM) supplemented with fetal bovine serum (10%) and penicillin (100 IU/ml) and streptomycin (100 μg/ml). The cell line was grown under a humidified 5% carbon dioxide (CO_2) atmosphere at 37 °C in an incubator (Form Direct Heat CO_2, Thermo Electron Corporation, Madrid, Spain) and the medium was changed every other day.

For experiments HepG2 cells (1.10^6 /ml) were seeded in each well of a 96-well culture plate and, after overnight incubation, a solution of different concentrations of gamma-decanolactone (10, 25, 50 and 100 μM) in dimethyl sulfoxide (DMSO) 1% was added to the cells in each well. Cells treated with DMSO 1% were used as negative control. After further incubation for 24 h, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay was performed to measure cell viability. Briefly, 10% of MTT solution (5 mg/ml in PBS) was added to each well. After incubation for 2 h at 37 °C, the MTT solution was removed and 100 μl of DMSO were added to dissolve the formazan crystals formed. Then, absorbance at 540 nm was determinated using a microplate reader (Fluo-Star Optima, BMG LABTECH, Offenburg, Germany). The percentage cell viability was calculated as [Absorbance (treatment)/Absorbance (negative control)] X 100 (Liu et al., 1997).

2.2. Computational studies (in silico)

2.2.1. Docking to the MAO isoforms

The two-dimensional structure of gamma-decanolactone ligand was obtained using Marvin JS tool (www.chemaxon.com) and converted into three-dimensional (3D) using Open Babel software (O'Boyle et al., 2011). The 3D structure of gamma-decanolactone was subjected to geometry optimization within the framework of density functional theory (Becke, 1993) using Gaussian basis set 6-31G* (Rassolov et al., 2001) in combination with B3LYP functional (Lee et al., 1988). To evaluate and understand how well ligand gamma-decanolactone binds to the molecular targets MAO-B and MAO-A, molecular docking studies were performed. The 3D-structure of the targets, human MAO-B (PDB ID:2V61) (Binda et al., 2007) and human MAO-A (PDB ID:2Z5X) (Son et al., 2008) were downloaded from Protein Data Bank.

The COACH-D webserver (Wu et al., 2018) performs prediction of protein-ligand binding pockets and residues by implementing a consensus among five methods: TM-SITE (Yang et al., 2013), S-SITE (Yang et al., 2013), FINDSITE (Brylinski and Skolnick, 2008), COFACTOR (Roy et al., 2012) and ConCavity (Capra et al., 2009). The ligand gamma-decanolactone is then docked into the predicted binding pockets using AutoDock Vina (Trott and Olson, 2010), a molecular docking algorithm. For each predicted binding pocket, up to 10 binding poses are generated, and the one that best matches the consensus prediction of the binding residues was then selected.

2.2.2. Prediction of physicochemical properties

In order to better understand the overall properties of the gamma-decanolactone, we also performed theoretical calculations using the Molinspiration program (Molinspiration Cheminformatics software, https://www.molinspiration.com/) to predict its physicochemical properties and ADME (absorption, distribution, metabolism and excretion) parameters and to compare these results with the reference MAO inhibitors (Lipinski et al., 1997).

2.2.3. Prediction of ability to cross the blood brain barrier (BBB)

Prediction of Blood Brain Barrier crosser was evaluated with the Online BBB Predictor (https://www.cbligand.org/BBB/) with the application of two algorithms, support vector machine (SVM) and AdaBoost, with four tipes of fingerprints: MACCS, Openbabel (FP2), Molprint 2D and PubChem (Liu et al., 2014).

2.3. In vivo model

2.3.1. Animals

Male swiss albino mice (weighting 22 ± 3 g) were housed in groups of 4 or 8 in standard Makrolon® cages (215 mm × 465 mm × 145 mm). The animals received standard laboratory chow (Scientific Animal Food and Engineering (SAFE), Augy, France) and tap water ad lib until the beginning of the experiments.

Both the housing and handling during experimentation were carried out according with the requeriments about animal experimentation established in the international and national legislation (Law 11.794 and Decree 6899 of the Brasilian Government, Guidelines of the Brazilian Council of Experimental Animals - CONCEA -, the European Directive 2010/63 /EU, the Spanish Royal Decrees (RD) RD 53/2013, RD 1386/2018, RD 118/2021, and the Spanish Order ECC / 566/2015).

The experiments were approved by the Committee on the Ethical Use of Animals of the UFRGS (authorization number 32940), and by the Informed Procedures of the Xunta de Galicia with the numbers: 15007AE / 09 / INV MED 02 / NER 02 / JAFG4, 15007AE / 09 / INV MED 02 / NER 02 / JAFG14, 15007AE / 09 / INV MED 02 / NER 02 / JAFG15, and Authorized Project (number 15012/2021/008).

All the experiments were carried out following the good laboratory practices and performed in an isolated room with 12/12 h light/dark cycles (08:00–20:00 h, light) at 22 ± 1 °C, at the same times of day, to avoid potential variations caused by circadian rhythms.

2.3.2. Drugs

The drugs gamma-decanolactone (GD) (300 mg/kg), reserpine (RES) (1.5 mg/kg), levodopa (LD) (100 mg/kg), benserazide (BZ) (25 mg/kg) and reactives sodium carboxymethylcellulose (NaCMC), tween 80 (TW) and acetic acid (CH_3COOH) were purchased from Sigma–Aldrich (USA) or Merck (Germany). All drugs were administered intraperitoneally (i.p.) in a volume of 10 ml/kg body weight. The experiments run between 08:00 and 15:00 h. Reserpine was dissolved in water with acetic acid (1%), the gamma-decanolactone was dissolved in TW 80 (5%) and LD + BZ was suspended in NaCMC (1% w/v). The doses were chosen based on previous studies conducted in mice and/or based on preliminary studies conducted in our laboratory (Matos et al., 2013; Pflüger et al., 2018a).

2.3.3. Pharmacological protocol

Animals (10–12 per group) were randomly allocated into six different treatment groups. This protocol involves the administration of reserpine (1.5 mg/kg) or vehicle (H_2O + CH_3COOH 0.1%) to the animals, 18:00 h before to starting of each trial. After this time, gamma-decanolactone (300 mg/kg) or vehicle (TW 5%) were administered and 30 min later, were administered the LD + BZ (100:25 mg/kg) or vehicle (NaCMC 1%). Sixty minutes after the last administration were evaluated the motor coordination, body temperature and the locomotor activity, as described below. A schematic diagram demonstrating the experimental design is show in Fig. 2.

2.3.4. Motor coordination test

Motor coordination of the animals was assessed following the technique described by Dunham and Miya (1957), with minor modifications. Mice were initially trained to remain on the rotarod apparatus (Rotamex 4/8, Columbus Instruments, OH, USA) based on their capacity to maintain their balance on the rotating bar (3 cm diameter) at constant velocity of 16 rpm. The mice able to remain on the rod longer than 180 s were selected and classified into six groups of four mice per group. The animals assigned to each experimental group received the treatment, mentioned above, or vehicle and 60 min later (at time 0), each mouse was placed on the rota-rod, to evaluate the time of permanency (spent) on the rotating bar and the percentage of animals that failed (cut-off time was 180 s). Complementary measurements were made, every 30 min (00:30, 01:00, 01:30 h) and, finally, one after 24:00 h.

2.3.5. Effect on body temperature

The rectal temperature of each mouse was measured at the time -18:00 h (basal temperature before reserpine administration) and at the times 00:00, 00:30, 01:00, 01:30 and 24:00 h after treatment of each group using a thermometer model 0331 (PanLab Instruments, Barcelona, Spain).

2.3.6. Locomotor activity

The locomotor activity in mice was performed using the open field test, following the protocol described by Rodríguez-Enríquez et al. (2020), with minor modifications. After receiving the treatments described above, the animals were placed in a black square box (100 × 100 × 30 cm^3), subdivided into 4 independent areas (50 × 50 × 30 cm^3) where each animal was placed independently. The evaluation of the locomotor activity was carried out from a room adjacent to the one containing the animal, using a video registration system. The behavior

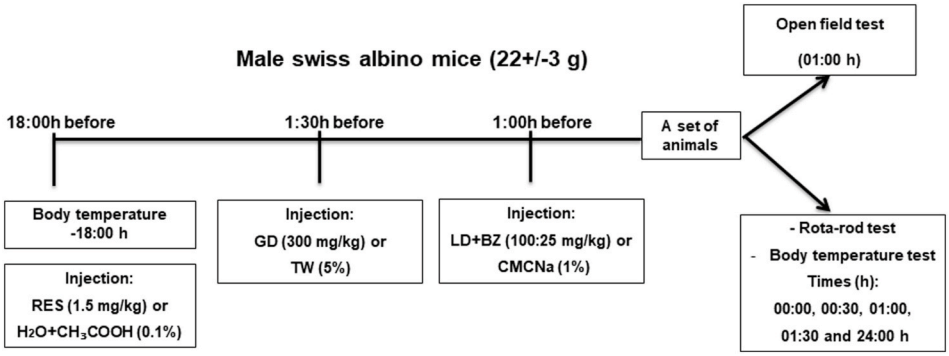

Fig. 2. Timeline representing the experimental design and times for treatments and evaluations of the different groups. RES: reserpine, H_2O + CH_3COOH (0.1%): water with acetic acid (0.1%), GD: Gamma-decanolactone, TW: Tween 80, LD + BZ: Levodopa plus Benserazide, CMCNa: Sodium Carboxymethylcellulose.

of the animals was captured with an analog camcorder (Sony DXC-107AP, Sony Corporation, Japan) suspended on the ceiling for the period of 1 h. The camera is connected to an adapter (Sony CMA-D2CE) that sends the signal to a monitor (Sony PVM-14M2E) and to two digitizing cards: i) an internal one located in a PCI slot of the computer (Picolo frame graber, Euresys, Liege, Belgium); ii) an external one with USB connection (DVC-USB, Dazzle, USA). The direct signal of the Picolo card is used by the video-computerized animal observation system (EthoVision V. 3.1.16, Noldus Information Technology, Wageningen, Netherlands). The EthoVision software locates the center of the animal, stores the data, and allows further analysis. The parameters evaluated were total distance moved (in cm/h), velocity (cm/s) and frequency of rearing.

2.4. Statistical analysis

Results are expressed as mean ± S.E.M. The graphical representation and statistical analysis were performed using GraphPad Prism V.5 program (GraphPad Software, Inc. San Diego, CA, U.S.A.). Statistically significant differences were determined by one-way ANOVA (treatment) followed by the Tukey's multiple comparison test or by two-way ANOVA (treatment-time) followed by Bonferroni test. Significant differences were determined with $P < 0.05$.

3. Results

3.1. In vitro

3.1.1. Inhibition of MAO

The results of MAO-A and MAO-B inhibition studies by gamma-decanolactone and the selectivity index are described in Table 1. Enzymatic assays revealed that gamma-decanolactone inhibits the activity of the MAO-B isoform, with an IC_{50} 55.95 ± 9.06 μM (Fig. 3), although this inhibition is much less potent than that of reference drugs such as selegiline. On the other hand, gamma-decanolactone has not been shown to inhibit the activity of the MAO-A isoform by demonstrating a selective profile against MAO-B.

3.1.2. Reversibility

The mode of inhibition (reversible or irreversible) produced by gamma-decanolactone on the MAO-B isoform was characterized. An effective dilution method was used, and an irreversible inhibitor as selegiline (IC_{50}= 0.017 ± 0.0019 μM) was taken as standard (Copeland, 2005; Gerlach et al., 1992). As shown in Fig. 4, gamma-decanolactone inhibits the activity of MAO-B in a partially reversible way.

3.1.3. Determination of gamma-decanolactone inhibition binding at human A_1, A_{2A}, A_{2B} and A_3 adenosine receptors

The gamma-decanolactone barely bound to any of the adenosine receptors evaluated in this study, since the percentages of inhibition were very low (Table 2A) at relatively high concentration (1–10 μM), compared to the Ki, in the nanomolar range, for the compounds used as standard reference drugs, as shown in Table 2B.

Table 1
IC_{50} values and MAO B selectivity index (SI) [IC_{50} (MAO A)]/[IC_{50} (MAO B)] for the inhibitory effects of GD on the enzymatic activity of human recombinant MAO isoforms expressed in baculovirus infected BTI insect cells.

Compound	hMAOA (μM) IC_{50}	hMAOB (μM) IC_{50}	S.I.
GD	#	55.95 ± 9.06	>1.79[&]
Selegiline	68.73 ± 4.21	0.017 ± 0.0019	4.043
Moclobemide	361.38 ± 19.37	#	<0.36[&]

Each IC_{50} value is the mean ± S.E.M. from three experiments (n = 3). [#]IC_{50}>100, at higher concentration compounds precipitate. [&]Values obtained under assumption that the corresponding IC_{50} against MAO-A or MAO-B is the highest concentration tested (100 μM). SI: hMAO-B selectivity index = IC_{50} (hMAO-A) /IC_{50} (hMAO-B).

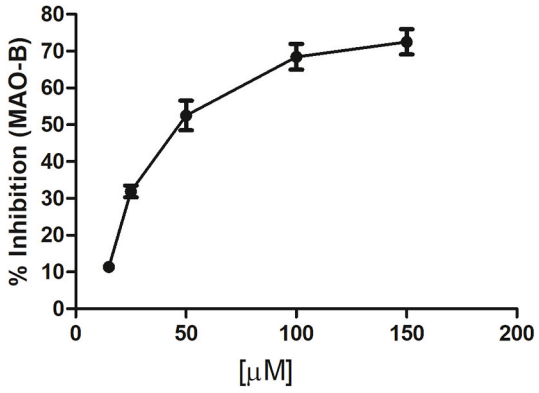

Fig. 3. Concentration-response curve of GD (15–150 μM) against percent inhibition of hMAO-B. Data are the mean ± S.E.M.

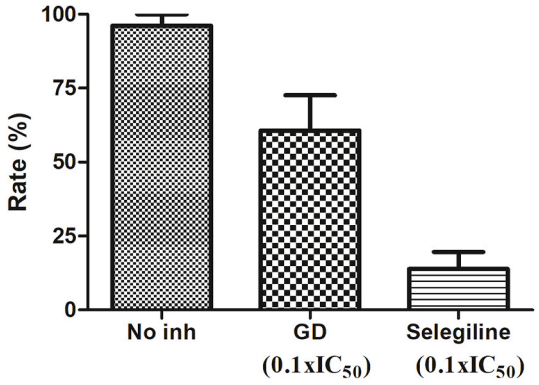

Fig. 4. Recovery of hMAO-B activity treated with GD and selegiline after 40 min preincubation and dilution from 10xCI_{50} to 0.1xIC_{50} and enzymatic activity of control (No inh). Data are the mean ± S.E.M.

Table 2A
Results of percentage of inhibition of GD in adenosine receptors.

	Receptor			
	hA1	hA2A	hA2B	hA3
Compounds	% Inhib. (1 μM)	% Inhib. (1 μM)	% Inhib. (10 μM)	% Inhib. (1 μM)
GD	9 ± 3	7 ± 3	17 ± 2	8 ± 2

3.1.4. Cytotoxicity of gamma-decanolactone

The cytotoxic effect of four gamma-decanolactone concentrations (10, 25, 50 and 100 μM) was evaluated by using the HepG2 cell line and the percentage of cell viability was measured as MTT reduction (Liu et al., 1997). As depicted in Fig. 5, just only the highest 100 μM

Table 2B
Affinity values (Ki) obtained and described in the literature for the compounds used to validate the assays.

Receptor	Compound	Ki (nM) obtained	Ki (nM) described	Reference
hA_1	XAC	14.3	29.1	Klotz et al. (1998)
hA_{2A}	CGS-15943	1.3	4.3	Fredholm et al., 2001
hA_{2B}	ZM 241385	12.2	32.0	Fredholm et al. (2001)
hA_3	MRS 1220	1.6	1.7	Gao and Jacobson, 2002

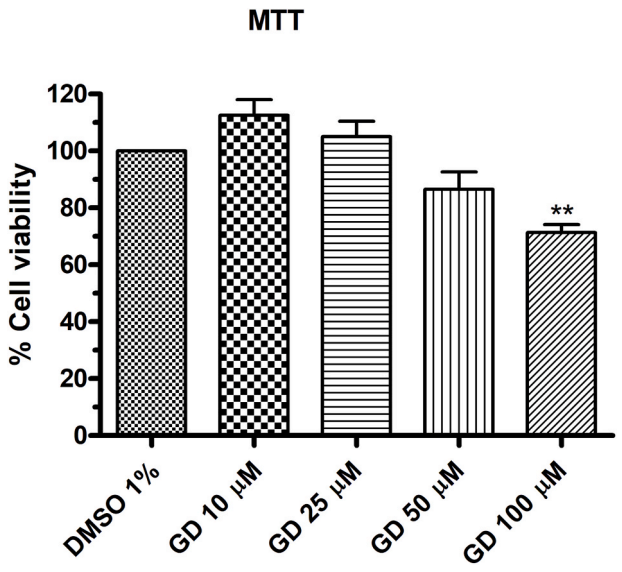

Fig. 5. Cytotoxic activity after 24 h incubation with GD at 10, 25, 50 or 100 μM on HepG2 cells. Cell viability was measured as MTT reduction and data were normalized as % of control treated with 1% DMSO. Results are expressed as mean ± S.E.M. from at least 3 different assays. (**P < 0.01), compared with control group using the one-way ANOVA followed by Tukey test.

concentration significantly decreased the viability of HepG2 cells (P < 0.01), thereby demonstrating that gamma-decanolactone at the lower concentrations used in this study does not have hepatotoxic effects.

3.2. Computational studies

3.2.1. Docking to the MAO isoforms

Molecular docking method is a rapid and efficient computational strategy to predict, examine and identify novel interactions between molecular targets and ligand molecules relevant to human diseases. In this work, we applied molecular docking method to understand preferential binding characteristics of ligand gamma-decanolactone (GD) to MAO-B isoform with respect to MAO-A. In both isoforms, the docking simulations revealed gamma-decanolactone binding close to the active site of the protein (Fig. 6). Docking experiments revealed that gamma-decanolactone exhibited a strong binding affinity to the hMAO-B complex in terms of binding free energy (-7.8 kcal/mol) compared to the hMAO-A complex (-6.1 kcal/mol).

The detailed molecular picture for protein-ligand interactions for ligand gamma-decanolactone against the two isoforms are shown in Fig. 7. The strong binding affinity noted for GD ligand against hMAO-B can be associated with higher number of residues participating in the binding compared to the hMAO-A isoform. The gamma-decanolactone ligand adopts a relatively compact conformation in the active site of hMAO-B, while we note a relatively extended conformation of the ligand in the hMAO-A isoform. In particular, for the hMAO-B protein we note oxolan-2-one group of ligand to be actively involved in noncovalent interactions with residues Y398 and Y435, which constitute the aromatic cage in the substrate cavity region. As reported in our recent study (Delogu et al., 2021) we note favourable interactions of gamma-decanolactone ligand with residue Y326 (related to substrate specificity) and with I199 that forms gate between the ligand entrance and substrate-binding regions.

3.2.2. Prediction of physicochemical properties

The values of predicted parameters, including lipophilicity, expressed as miLogP, molecular weight (MW), topological polar surface area (TPSA), number of hydrogen donors (nON), number of hydrogen bond acceptors (nOHNH), molecular volume (MV), number of rotatable bonds (Nrotb), and the number of violations of the rule of five (Lipinski et al., 1997) are presented in Table 3.

It is important to highlight that gamma-decanolactone possess miLogP value compatible with those required to cross membranes. miLogP values in the −0.4 to +5.6 range are acceptable and gamma-decanolactone demonstrated a miLogP in this range (miLogP = 2.12), very similar to that obtained with the reference drugs.

TPSA, described to be a predictive indicator of membrane penetration, was found to be positive for gamma-decanolactone (TPSA = 26.30) although it has a much higher value than the reference drugs selegiline (TPSA = 3.24) and rasagiline (TPSA = 12.03), but much lower than that obtained with safinamide (64.36) (Ertl et al., 2000).

The molecular weight of gamma-decanolactone (170.25) is the lowest of all the drugs evaluated. In contrast, the highest molecular weight corresponded to safinamide (302.25). The results obtained with the other drugs evaluated are between the two mentioned drugs, with values of 171.24 and 187.29 for rasagiline and selegiline, respectively.

The results obtained for the H-bond acceptors prediction, show that the value obtained with gamma-decanolactone was two, and this value is the double that the obtained with selegiline (1) or rasagiline (1), but is

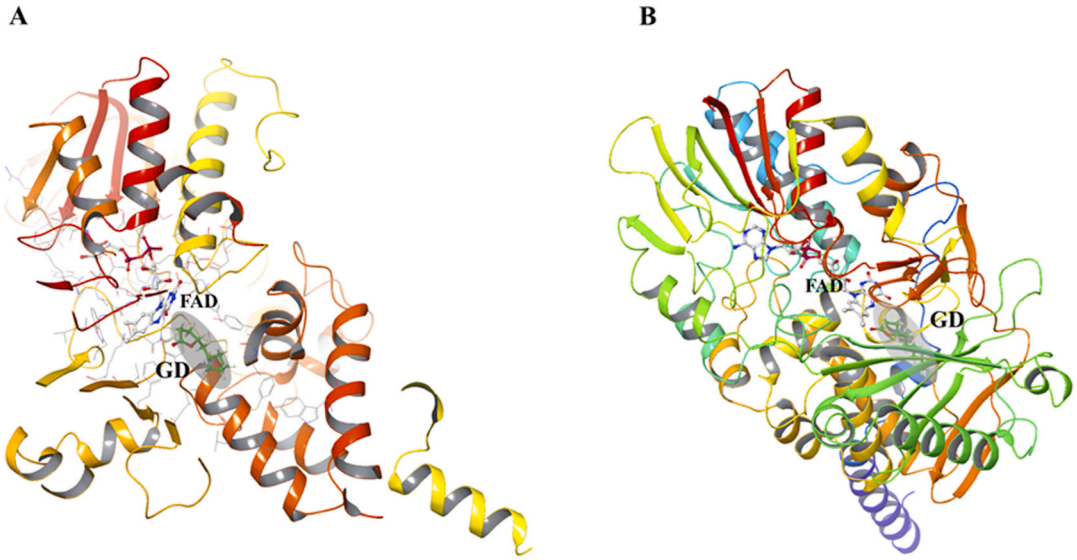

Fig. 6. Predicted protein-ligand complex structures. GD ligand in complex with human-MAO-B isoform is shown in (A) and with human MAO-A protein in (B).

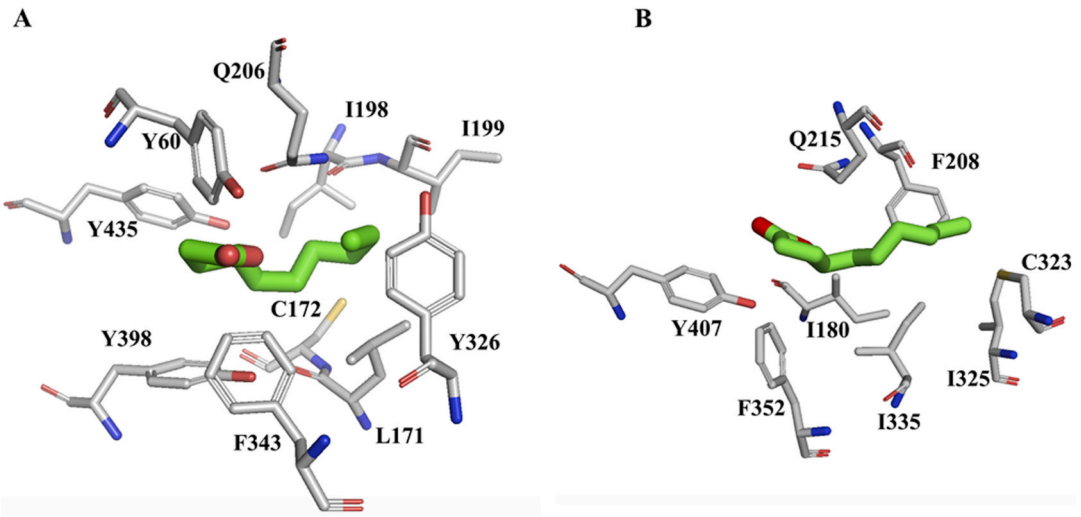

Fig. 7. Protein-ligand interactions. Gamma-decanolactone (GD) ligand in complex with human MAO-B isoform is shown in (A) and with human MAO-A protein in (B). The GD is shown in green and key protein residues are shown in grey with single letter aminoacid code. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Physicochemical properties of GD and the MAOI-B reference drugs.

Compound	miLogP	MW	TPSA	H-bond acceptors	H-bond donors	MV	Nrotb	Lipinski's
GD	2.12	170.25	26.30	2	0	180.76	5	0
Selegiline	2.64	187.29	3.24	1	0	202.64	4	0
Rasagiline	2.10	171.24	12.03	1	1	175.10	2	0
Safinamide	2.91	302.35	64.36	4	3	279.04	7	0

miLogP – expressed as the logarithm of the octanol/water partition coefficient. MW – molecular weight. TPSA – topological polar surface area. H-bond acceptors and donors - Number of hydrogen bond acceptors (nON) and donors (nOHNH). MV– molecular volume. Nrotb – number of rotatable bonds. Lipinski's –number of violations of the rule of five.

the half that the obtained with safinamide (4).

In the H-bond donors the results for gamma-decalactone and selegiline were identical (0), the rasagiline value was 1 and for the safinamide, again the highest value was obtained (3).

The molecular volume for gamma-decanolactone (180.76) was slightly higher that the obtained with rasagiline (175.10) but lower tha the obtained with selegiline (202.64) or safinamide (279.04).

The more flexibility molecules were safinamide, with seven rotatable bonds and the gamma-decanolactone with five rotatable bonds, respectively. The greatest stiffness was observed with rasagiline, with two rotatable bonds, and selegiline with four rotatable bonds. The molecular flexibility is a good indicator of oral bioavailability of drugs (Veber et al., 2002).

In addition, neither gamma-decanolactone nor any of the reference molecules violate the Lipinski's rule of the fives (Lipinski et al., 1997), so called because the reference limit values are five or a multiple of five. The gamma-decanolactone has a miLogP < 5, the molecular weight $<$ 500, the number of hydrogen bond acceptors < 10, and the number of hydrogen bond donors < 5. With these results, gamma-decanolactone exhibits adequate physicochemical properties to become a good drug.

3.2.3. Prediction of ability to cross the blood brain barrier (BBB)

The blood-brain barrier crosser properties for gamma-decanolactone and the IMAO-B reference standard drugs, selegiline, rasagiline and safinamide, were evaluated using the Online BBB Predictor with the two algorithms, the support vector machine (SVM) and AdaBoost, with four fingerprints: MACCS, Open Babel (FP2), Molprint 2D, and PubChem. We have used different methods to ensure the validity of the results. The obtained results show that gamma-decanolactone and the reference drugs have values compatible with those required to cross the blood-brain barrier as show in the Table 4.

Table 4
(A and B): Prediction to cross the Blood Brain Barrier (BBB).

(A)	AdaBoost algorithm			
	Fingerprint			
Compound	MACCS	Open Babel (FP2)	Molprint 2D	PubChem
GD	7.036	11.288	2.931	10.225
Selegiline	7.464	10.964	8.447	9.465
Rasagiline	8.614	10.405	7.428	10.997
Safinamide	3.128	5.454	10.783	-1.037
(B)	SVM algorithm			
	Fingerprint			
Compound	MACCS	Open Babel (FP2)	Molprint 2D	PubChem
GD	0.052	0.212	0.314	0.465
Selegiline	0.110	0.138	0.347	0.239
Rasagiline	0.090	0.216	0.330	0.331
Safinamide	0.067	0.143	0.465	0.127

AdaBoost and SVM algorithms: The threshold score for cross the BBB (BBB+) or not cross (BBB-) is 0, except for SVM MACCS that is 0.02. AdaBoost scale: MACCS (-15.00 – +15.00), Open Babel (FP2) (-40.00 – +40:00), Molprint 2D (-20.00 - +20.00), PubChem (-30.00 – +30.00). SVM scale: MACCS (-0.80 – +0.50), Open Babel (FP2) (-2.00 – +2:00), Molprint 2D (-1.00 - +2.00), PubChem (-2.00 – +1.00).

The results obtained with Adaboost algorithm were similar, in all fingerprints, for gamma-decanolactone and the two classical MAO-B inhibitors, selegiline and safinamide, with values > 7, except in the Molprint 2D algorithm, where the gamma-decanolactone exhibited a very low value (2.931). Conversely, the more rencently MAO-B inhibitor commercialized, the safinamide, show a very low value in the three of four fingerprints examinated except, in contraste with gamma-

decanolactone, in the Molprint 2D fingerprint value, where has a maximun value of 10.783. Highlight the paradox of the negative value obtained with safinamide with the PubChem fingerprint.

In any case, the values obtained with gamma-decanolactone with this algorithm predict its ability to pass through the BBB.

In contrast to the previous algorithm, in the SVM algorithm, the values of gamma-decanolactone and the reference drugs are more similar and, in all cases, with positive values that predict their ability to pass through the blood-brain barrier.

3.3. In vivo assays

3.3.1. Motor coordination

The rota-rod test was used to assess motor activity coordination in experimental animals. In non reserpinized animals treated with gamma-decanolactone, at the doses tested, or in the TW vehicle group not effect was observed in motor coordination.

Reserpinized animals showed a significant increase in ataxia at all times measured ($P<0.001$) compared to the control group, except for 24 h. A marked ataxia was shown at 01:30h (57.88 ± 21.39 s), proving to decrease motor coordination and to be an effective model. Treatment with LD + BZ partially reversed ataxia after 01:00 h (158.70 ± 14.49 s, $P<0.001$) and 01:30 h (139.60 ± 15.96 s, $P<0.005$), as did gamma-decanolactone alone after 01:00 h and 01:30 h (128.80 ± 22.30 s, $P<0.05$ and 126.00 ± 20.18 s, $P<0.05$; respectively, compared to the reserpine group). Concomitant treatment of gamma-decanolactone with LD + BZ, at 01:30 h showed a potentiation of the protective effect (162.90 ± 12.89 s, $P<0.001$) compared to the reserpine group. This treatment allowed the animals to return to their normal state, demonstrating that there is no more difference between this group and the non reserpinized group (control group). The percentage of animals treated with gamma-decanolactone plus LD + BZ that failed at the 24 h is very low and similar to vehicle treated group whereas, at the same time of evaluation, the groups treated alone with gamma-decanolactone or LD + BZ have a percentage of fails about to 40% (Fig. 8 and Fig. 9). These results show a potentiation of pharmacological effects with the association of gamma-decanolactone plus LD + BZ.

3.3.2. Body temperature

No changes in the body temperature were induced by gamma-decanolactone in non reserpinized mice. Reserpinized animals showed a marked decrease in body temperature at all times measured ($P<0.001$), except after 24 h, compared to non reserpinized mice. The lowest temperature (32.86 ± 0.54 °C) was reached in the first measurement (00:00 h) at 18 h after the administration of reserpine. Only treatment with gamma-decanolactone alone facilitated the significant reversal of hypothermia in all measured times ($P<0.01$), except at 24 h, compared to the reserpinized animals. At the dose tested, no changes in the body temperature of the animals were induced by LD + BZ alone or associated with gamma-decanolactone. Almost all animals practically returned to normal body temperature after 24 h, as shown in Fig. 10.

3.3.3. Open field test

Evaluation of locomotor activity in the reserpinized animals showed a significantly marked reduction of the total distance moved (in cm), speed (cm/s) and percentage of rearing, compared to the control group treated with the vehicle (H_2O + CH_3COOH) ($P<0.001$). This finding confirms hypokinesia due to decreased dopamine in the nigrostriatal pathway, which is caused by the reserpine a the dose utilized. The treatment with gamma-decanolactone alone (300 mg/kg) or LD + BZ alone (100:25 mg/kg) and the co-treatment with both associated drugs, did not reverse the hypokinesia caused by reserpine ($P > 0.05$) in the tested dose (Fig. 11). Also, not changes were manifested in the other parameters analyzed in this study, velocity, and rearing (data not shown).

4. Discussion

The neuroprotective and anti-convulsant effects of gamma-decanolactone in the *in vivo* and *in vitro* studies have been previously reported (Coelho et al., 1997; De Oliveira et al., 2008; Pflüger et al., 2016, 2018a, 2018b, 2018b). The potential interrelation between epilepsy and neurodegenerative diseases, as Parkinson's disease, is present in the scientific literature (Deuel et al., 2019; Gruntz et al., 2018; Murata et al., 2007; Scorza et al., 2018a; Son et al., 2016; Vercueil, 2000).

MAO-B inhibitors are a well-known and effective group of drugs for the Parkinson treatment. The MAO-B inhibitors reduce the enzymatic degradation of dopamine by MAO in neural and glial cells, thus leading to an increase of dopamine in nigrostriatal dopaminergic pathway that improving motor symptoms (locomotor activity and motor coordination) in patients with Parkinson's disease (Mallajosyula et al., 2008). MAO-B inhibitors also increased dopamine in anothers dopaminergic pathways implicated in regulation of body temperature, inhibition of prolactine (PRL) secrection, etc. In general, MAO-B inhibitor compounds have a good safety and tolerability profile (Olaya et al., 2019; Riederer and Müller, 2018), which makes them interesting as group to search for new MAO-B inhibitors to be used in the treatment of patients with

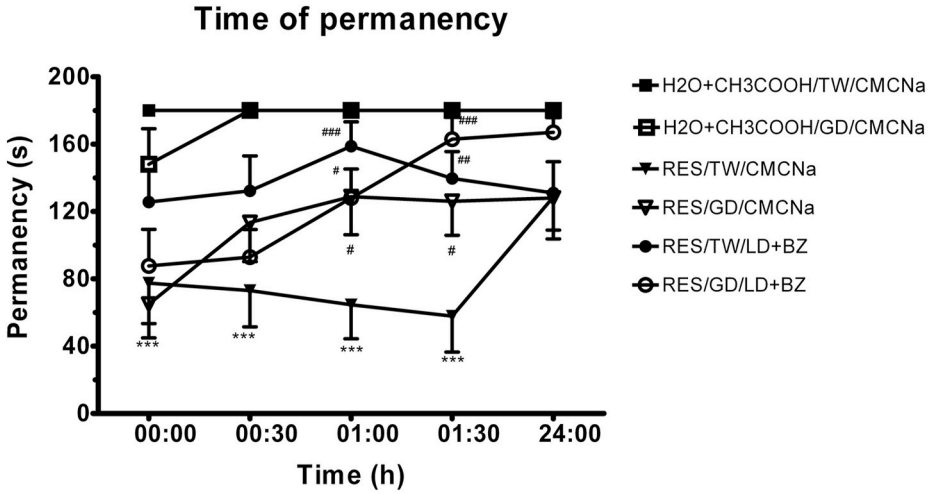

Fig. 8. Time of permanency of the animals in the rotating-rod. (***$P \leq 0.001$) compared to the group H_2O + CH_3COOH/TW/CMCNa and ($^{\#}P \leq 0.05$, $^{\#\#}P \leq 0.01$, $^{\#\#\#}P \leq 0.001$) compared to the RES/TW/CMCNa group. Cut off time 180 s. Values represent the mean ± S.E.M.

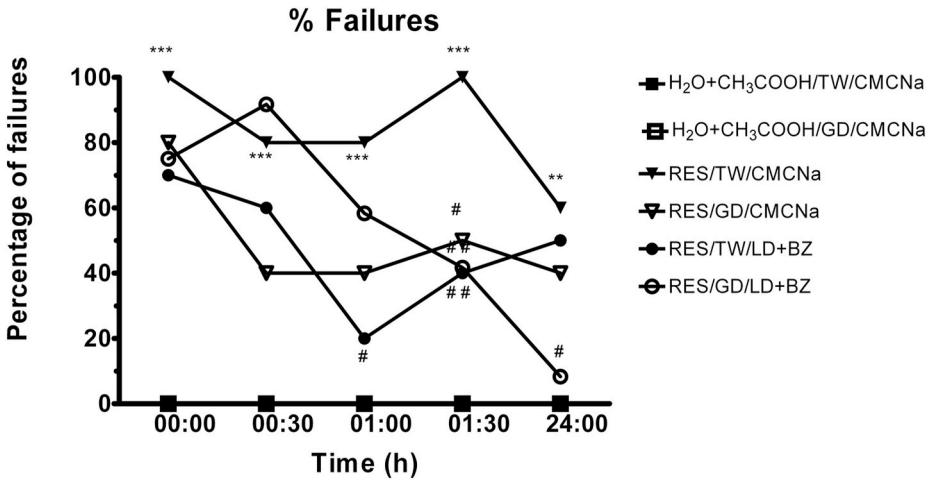

Fig. 9. Percentage of animals that failed in the rotating-rod. (**P ≤ 0.01, ***P ≤ 0.001) compared to the group H_2O + CH_3COOH/TW/CMCNa and ($^{\#}$P ≤ 0.05, $^{\#\#}$P ≤ 0.01) compared to the RES/TW/CMCNa group. Values represent the mean ± S.E.M.

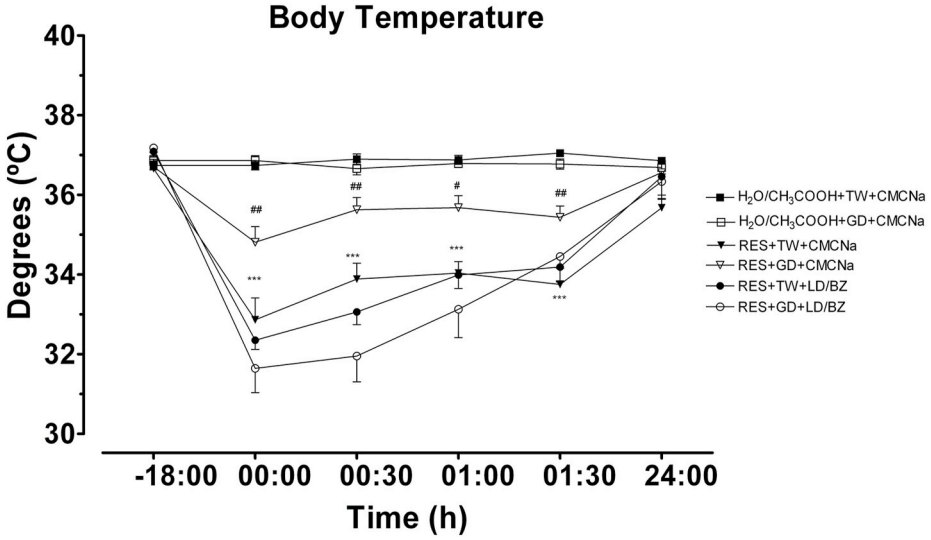

Fig. 10. Changes in the body temperature of the animals. (***P≤ 0.001) compared to the group H_2O + CH_3COOH/TW/CMCNa and ($^{\#}$P ≤ 0.05, $^{\#\#}$P ≤ 0.01) compared to the RES/TW/CMCNa group. Values represent the mean ± S.E.M.

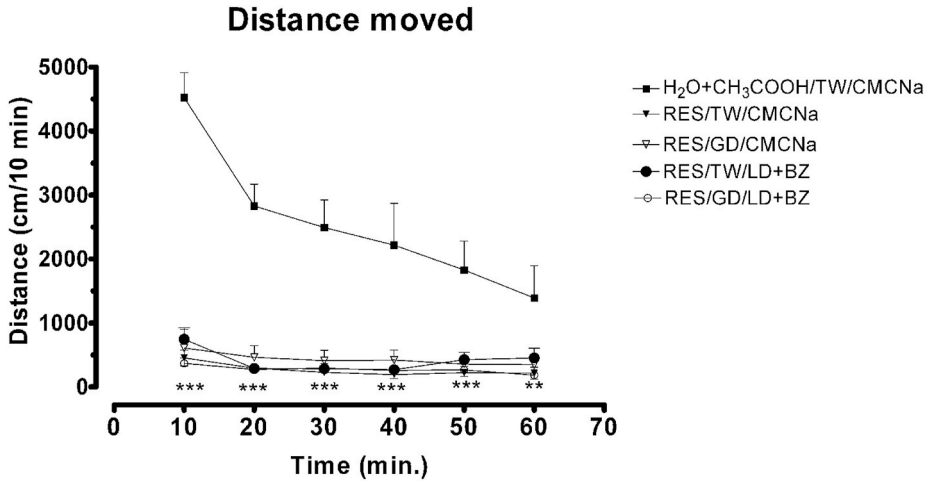

Fig. 11. Total distance travelled every 10 min, for 1 h of observation of the mice in the open field test. **P≤ 0.01, ***P ≤ 0.001 compared to the vehicle control group H_2O + CH_3COOH/TW/CMCNa. Values represent the mean ± S.E.M.

Parkinson's disease.

In the current study, we confirmed the potential neuroprotective effects of this monoterpene compound, the gamma decanolactone, in neurodegenerative diseases, as Parkinson's disease. The gamma-decanolactone resulted a selective MAO-B inhibitor, partially reversible, in the MAO *in vitro* studies performed.

The molecular docking analysis of the binding of gamma-decanolactone revealed better affinity characteristics with hMAO-B in terms of binding energy and number of computed interactions compared to hMAO-A protein. The trend in the computed binding energies of gamma-decanolactone ligand with the two hMAO isoforms matched closely with the trend in the experimental IC_{50} values. A detailed analysis revealed higher number of non-covalent interaction of gamma-decanolactone with hMAO-B than with hMAO-A. The gamma butyrolactone moiety of gamma-decanolactone exhibited non-covalent interactions with three tyrosyl residues (Y60, Y398 and Y435) located in the substrate binding site. While, the other aliphatic chains of gamma-decanolactone ligand are surrounded by array of isoleucline residues (I171, I198, I199), C172, Q206, and Y326, thus resulting in strong binding affinity to hMAO-B protein. Indeed the importance of Y326 residue in substrate specificity and the action of I199 as a gate between the ligand and substrate entrance has been reported in a recent study (Delogu et al., 2021). Altogether, the determined residues in this study could play an important part towards potent inhibition, high selective index, and strong binding affinity of gamma-decanolactone towards hMAO-B than hMAO-A protein.

A basic condition of any compound to act on CNS neurodegenerative diseases is to be able to cross the blood brain barrier and penetrate in the brain.

In the computational studies with Molispiration, the gamma-decanolactone exhibited physicochemical values compatible with those required to cross membranes, good bioability and does not violate the rule of five of Lipinski's, thus gamma-decanolactone exhibits adequate properties to become a good drug.

In the evaluation of prediction to cross the BBB by the gamma-decanolactone, with the Online BBB Predictor study, the gamma decanolactone exhibited capacity to cross the BBB in all cases. These predictive results are in accordance with the previously obtained results *in vivo* in the anticonvulsive studies with gamma-decanolactone, and with the *in vivo* results obtained in this paper. All of this results reliably demonstrates the ability of the gamma-decanolactone to cross the BBB.

In addition to Parkinson's disease, the MAO-B inhibition also is potentially beneficious in the most predominant neurodegenerative disease, the Alzheimer's disease (Cai, 2014; Park et al., 2019; Schedin-Weiss et al., 2017).

These results together give an idea of the potential interest of gamma-decanolactone to treat two of the most prominent neurodegenerative diseases. In further studies we will board the evaluation of the gamma-decanolactone in animal models of the Alzheimer's disease.

Also, in the cytotoxicity studies, only the highest concentration of the gamma-decanolactone evaluated (100 μM) significantly decreased the viability of HepG2 cells. All other concentrations were well tolerated for the cells, including the 50 μM concentration, the value obtained in the IC_{50} of the MAO-B activity, expressing the safety of this compound.

The reduction of locomotor activity in mice by reserpine is frequently used as a model of motor disturbances in Parkinson's disease (Tadaiesky et al., 2006). The hypokinesia - akinesia, the increase in ataxia (motor incoordination) and the hypothermia caused by reserpine in mice occur due to the blocking of the vesicular monoamine transporter (VMAT2) that produces an intense and prolonged decrease of dopamine in all dopaminergic nerve terminals, including the nigrostriatal pathway (Freitas et al., 2016) implicated in the motor disorders in the Parkinson's disease. Several clinically used antiparkinsonian drugs (e.g. L-DOPA, alone or associated with periferically descarboxilase inhibitors -carbidopa, benserazide-, dopamine receptor agonists -e.g. apomorphine, pramipexol-, the antiviral amantadine and/or the anticholinergic trihexyphenidyl) were tested in this model, suggesting it has predictive validity for discover new potential antiparkinsonian drugs (Menzaghi et al., 1997; Olaya et al., 2019).

In the motor coordination test, both gamma-decanolactone and levodopa plus benserazide (LD + BZ) association partially reversed ataxia induced by resepine after the period 01:00 h. Concomitant treatment of gamma-decanolactone with levodopa plus benserazide showed a potentiation of the protective effect in mice. These data can be explained by the action of gamma-decanolactone inhibiting the MAO-B activity in a partially reversible way, as demonstrated previously in this work, increasing dopamine levels and, consequently, improving the motor coordination of mice in the rotarod.

Clasically, the dopaminergic agonists and the dopamine/norepinephrine neuronal transporter inhibitors are associated with increases in the body temperature in animals whereas, conversely, the dopaminergic antagonists are associated with decreased temperature (Lin, 1979; Hasegawa et al., 2005). Also, according to Leon (2004), pro-inflammatory cytokines such as TNF-α, interleukins (ILs) and interferon-gamma have been shown to induce or modulate hypothermia. The MAO-B inhibitory activity shown by the gamma-decanolactone in this study, with the subsequent increased of dopamine in the synaptic space, together with the already proven capacity of gamma decanolactone to decrease the expression of TNF-α in the lipopolysaccharide (LPS) model in microglial cells (Pflüger et al., 2016), can explaind the partially reversion of hypothermia found in this study.

Gamma-decanolactone or levodopa plus benserazide alone and the co-treatment with both drugs did not reverse the hypokinesia induced by reserpine in mice evaluated in the open field test. No increases in distance travelled, velocity or rearing were observed *vs* reserpine treated group. Possibly, the effects observed in this test, are related to the sedative and hypnotic activities of gamma-decanolactone, corroborating with the results of other studies (Coelho de Souza et al., 1997; Viana et al., 2007; Pflüger et al., 2018b). The sedative effects on the CNS of other monoterpene compounds and some lactones have been reported by Okugawa et al. (1996).

Adenosine A_{2A} receptor antagonists represent a new path to follow in the symptomatic treatment of Parkinson's disease through a non-dopaminergic mechanism, a hypokinetic movement disorder (Jaiteh et al., 2018). It is known that adenosine A_{2A} and dopamine D_2 receptors are functionally antagonistic, since the A_{2A} receptor antagonist may have a similar effect on the motor control that the D_2 receptor agonists for the treatment of Parkinson's disease (Mori, 2014). Knowing the involvement of adenosine in the treatment of Parkinson, we investigated the binding of gamma decanolactone to adenosine receptors (A_1, A_{2A}, A_{2B} and A_3); however, gamma-decanolactone practically did not bind to any of the adenosine receptors evaluated in this study (percentage of inhibition below 20%), corroborating with another study carried out by our research group, in which no changes in the expression of the adenosine A_1 receptor were detected in the hippocampus of mice treated with gamma decanolactone in the western blotting test (Pflüger et al., 2020). In this same study, when we evaluated the action of gamma-decanolactone in two behavioral models of epilepsy, using adenosine A_1 and A_{2A} receptor antagonists. Gamma-decanolactone demonstrated not to modulate the adenosine A_{2A} receptor antagonist, but, in contrast, modulated the adenosine receptor antagonist A_1, so further studies are needed to clarify the involvement of gamma-decanolactone in adenosine receptors.

5. Conclusion

In summary, in this work we have demonstrated that the monoterpene compound gamma-decanolactone acts as MAO-B inhibitor, in a partially reversible way, with neuroprotective profile.

Highlighting also, that gamma-decanolactone adheres to Lipinski's rule of five and has capacity to cross the blood-brain barrier. All these properties put together suggest that it has desirable properties for the

development of a possible new drug candidate or scaffold for the design of new drugs with activity in the central nervous system.

The gamma-decanolactone crosses the blood-brain barrier in *in vivo* experiments and can partially reverse the ataxia and hypothermia caused by reserpine but fails to reverse the hypokinesia in mice.

Additional studies that can elucidate the mechanism of action of gamma-decanolactone, with identification of its main molecular targets, are of great importance for the confirmation of this compound as a potential candidate for antiparkinsonian drug. Also, the future evaluation of gamma decanolactone in the Alzheimer animal models is coherent with their partially reversible MAO-B inhibitor profile.

CRediT authorship contribution statement

Pricila Pflüger: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Writing – original draft. **Patrícia Pereira:** Conceptualization, Methodology, Validation, Formal analysis, Resources, Supervision. **María I. Loza:** Resources, Validation. **José Brea:** Resources, Investigation, Validation. **Dolores Viña:** Methodology, Resources, Investigation, Validation, Formal analysis, Writing – review & editing. **Amit Kumar:** Methodology, Software, Resources, Formal analysis, Writing – original draft. **José A. Fontenla:** Conceptualization, Methodology, Resources, Investigation, Validation, Formal analysis, Writing – review & editing, Supervision.

Declaration of competing interest

None.

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