International Journal of Pharmaceutics: X 8 (2024) 100296

Contents lists available at ScienceDirect

International Journal of Pharmaceutics: X

journal homepage: www.sciencedirect.com/journal/international-journal-of-pharmaceutics-x

Design of an innovative nanovehicle to enhance brain permeability of a novel 5-HT6 receptor antagonist

María Javiera Alvarez-Figueroa [a,*,1], Francisco Nuñez-Navarro [a], Gonzalo Recabarren-Gajardo [a,b], José Vicente González-Aramundiz [a,c,*,1]

[a] Departamento de Farmacia, Facultad de Química, Pontificia Universidad Católica de Chile, Santiago, Chile
[b] Centro Interdisciplinario de Neurociencias, Pontificia Universidad Católica de Chile, Santiago, Chile
[c] Centro de Investigación en Nanotecnología y Materiales Avanzados "CIEN-UC ", Pontificia Universidad Católica de Chile, Santiago, Chile

ARTICLE INFO

Keywords:
Lipid nanocapsules
PAMPA-BBB
Brain permeability
Arylsulfonylindoles
5-HT6 receptor

ABSTRACT

An innovative nanovehicle based on lipid nanocapsules (LNC) was designed to facilitate the passage of a new 5-HT_6 receptor antagonist, namely PUC-10, through the blood-brain barrier. PUC-10 is a new synthetic *N*-arylsulfonylindole that has demonstrated potent 5-HT_6 receptor antagonist activity, but it exhibits poor solubility in water, which indicates limited absorption. The lipid nanocapsules designed had a nanometric size (53 nm), a monomodal distribution (PI<0.2), a negative Z potential (−17 ± 7 mV) and allowed efficient PUC-10 encapsulation (74 %). Furthermore, the LNC demonstrated to be stable for at least 4 weeks at 4 °C (storage conditions), for at least 4 h in DMEM at pH 7.4, and for 18 h in water with 5 % DMSO, with both latter conditions maintained at 37 °C. They also demonstrated that cell viability was not affected at the different concentrations studied. Finally, *in vitro* studies that simulate the blood brain barrier (PAMPA-BBB) demonstrated that the nanoencapsulation of PUC-10 promoted their penetration through the blood-brain barrier, with a calculated permeability of 1.3×10^{-8} cm/s, compared to the null permeability exhibited by non-nanoencapsulated PUC-10.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is an important neurotransmitter that regulates numerous physiological functions, including thermoregulation, sexual and aggressive behavior, learning and memory, endocrine and gastrointestinal functions, and food intake, among others (Jones et al., 2020). To perform these various tasks, the neurotransmitter 5-HT interacts with multiple receptor subtypes, including the 5-HT_6 receptor (5-HT_6R), which is expressed almost exclusively in the central nervous system (Monsma Jr. et al., 1993). 5-HT_6R is involved in several physiological functions, such as learning, memory, and neurodevelopment, as well as in diverse pathological states, such as eating disorders, anxiety, depression, addictive behavior, schizophrenia, epilepsy, and Alzheimer's disease (Arrieta-Rodríguez et al., 2021). 5-HT_6 receptor antagonists have demonstrated procognitive behavioral effects, making them potential cognitive enhancers in conditions associated with cognitive impairment (González-Vera et al., 2017; Grychowska et al., 2016; Lacivita et al., 2017). Thus, in recent years, studies have begun to be carried out with healthy volunteers to evaluate the safety, tolerability and efficacy of different compounds with 5-HT_6 antagonist activity to assess whether it is feasible for them to be approved as drugs (Fernandez et al., 2023; Li et al., 2021).

Based on the interest in these functions and their potential applications, the design, synthesis, and pharmacological evaluation of several *N*-arylsulfonylindole derivatives, which demonstrate moderate to high affinity and an antagonistic profile towards the 5-HT_6 receptor, have been previously reported (Ramírez, 2013; Vera et al., 2016). Among these derivatives, 2-(4-(2-methoxyphenyl)piperazin-1-yl)-1-(1-(naphthalen-1-ylsulfonyl)-1*H*-indol-3-yl)ethan-1-ol (**PUC-10**) stood out as the most active in the series (see Fig. 1).

PUC-10 is a synthetic molecule with a high molecular weight of 541.66 g/mol, is nonpolar and has poor water solubility. These properties suggest potential challenges in the absorption of this compound (Pajouhesh and Lenz, 2005; Rankovic, 2015). Thus, although PUC-10 exhibits powerful antagonism on the 5-HT_6 receptor, its pharmacokinetic properties limit its potential to become a drug.

* Corresponding authors at: Facultad de Química y de Farmacia, Escuela de Farmacia, Pontificia Universidad Católica de Chile, Vicuña Mackena 4860, CP:7820436, Macul. Santiago, Chile.
E-mail addresses: mjalvare@uc.cl (M.J. Alvarez-Figueroa), jvgonzal@uc.cl (J.V. González-Aramundiz).
[1] These authors contributed equally to this work and should be considered joint corresponding authors.

https://doi.org/10.1016/j.ijpx.2024.100296
Received 8 August 2024; Received in revised form 2 October 2024; Accepted 20 October 2024
Available online 21 October 2024

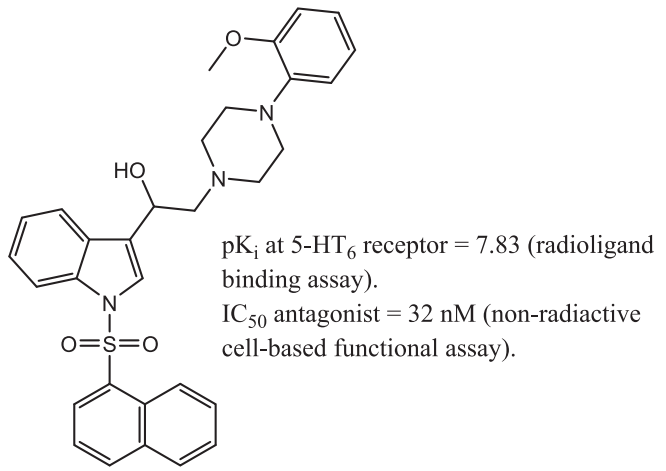

Fig. 1. Structure of the 5-HT$_6$ receptor antagonist 2-(4-(2-methoxyphenyl) piperazin-1-yl)-1-(1-(naphthalen-1-ylsulfonyl)-1H-indol-3-yl)ethan-1-ol (**PUC-10**) (Vera et al., 2016).

The PAMPA artificial permeability assay is widely used to evaluate the activity of candidate drug molecules since it is a relatively low-cost, rapid, robust and high-performance technique that very reliably predicts the absorption of drugs through passive diffusion mechanisms, a mechanism that explains the absorption of more than 90 % of drugs (Williams et al., 2022). Specifically, PAMPA studies simulating the blood-brain barrier (PAMPA-BBB) have demonstrated a very high *in vitro-in vivo* correlation (77 %) using rodents as *in vivo* models (Kato et al., 2023). The PAMPA-BBB assay, by assessing non-cellular passage, does not assess active flux of molecules, however, most drugs that diffuse through the CNS do so passively, making this assay a good predictor of molecular absorption across the blood-brain barrier (Kato et al., 2023).

Therefore, in this research we studied one of the strategies, namely nanoencapsulation, that allows increasing the solubility of certain molecules and promoting their penetration through biological barriers. Nanometric-sized systems allow increasing the effective absorption surface, favouring passage through membranes and therefore, increase the bioavailability of the nanoencapsulated molecule (Jaudoin et al., 2021; Pateiro et al., 2021; Valdivia-Olivares et al., 2023).

Specifically, the incorporation of PUC-10 into a lipid nanocapsule was studied. Lipid nanocapsules are nanoparticles composed of an oily core and a surfactant shell, making them excellent vehicles for encapsulating lipophilic molecules. Their size can be tuned by modifying the oil/surfactant ratio and they generally have sizes ranging from 20 to 100 nm (Rolley et al., 2021). LNC are produced through a temperature-induced phase inversion that follows the formation of an oil/water microemulsion containing an oily fat, an aqueous phase, a lipophilic surfactant, and a nonionic hydrophilic surfactant (Alvarez-Figueroa et al., 2022; Moura et al., 2020). Unlike most nanovehicles, the LNC preparation is free of organic solvents, which prevents eventual toxicity due to the presence of traces of organic solvents (Heurtault et al., 2002).

Due to the problems identified with PUC-10, this research aimed to design a suitable and biocompatible nanovehicle that could incorporate this molecule into its core and promote its passage through the blood-brain barrier. This was evaluated using a method that correlates very well with *in vivo* studies: PAMPA-BBB.

2. Materials and methods

2.1. Reagents

2-(4-(2-methoxyphenyl)piperazin-1-yl)-1-(1-(naphthalen-1-ylsulfonyl)-1*H*-indol-3-yl) than-1-ol (PUC-10) was obtained from "*Laboratorio de Síntesis de Heterociclos Bioactivos*", Professor Gonzalo Recabarren.

Lipoid P75® was obtained by LipoidGmbH, Germany. Labrafac® PG and Labrafil® M 1944 CS were purchased from Gattefossé (France). Chitosan HCl, Kolliphor HS and n-dodecane were obtained by Sigma-Aldrich (Germany). Sodium chloride, acetonitrile, sodium acetate and sodium dodecyl sulfate were purchased from Merck (Germany). Sodium thiopental was obtained by Rotexmedica (Germany). Porcine polar lipid was purchased from Avanti polar lipids (USA).

2.2. PUC-10 synthesis

PUC-10 was synthesized as previously described (Vera et al., 2016). Briefly, acylation of commercial indole was performed in a two-step sequence, starting from a premixed solution of heterocycle and anhydrous zinc chloride, to which methyl magnesium bromide was quickly added to provide the corresponding magnesium salt. This salt undergoes an *in situ* transmetallation reaction with the zinc chloride previously added. Then, the zinc salt was acylated with bromoacetyl chloride under inert atmosphere to afford bromoacetylindole. The haloketone was further reacted with naphthalene-1-sulfonyl chloride under basic conditions to afford the corresponding 2-bromo-1-(1-(naphthalen-1-ylsulfonyl)-1*H*-indol-3-yl) ethan-1-one. The prepared *N*-protected bromoketone was subjected to bromide displacement in basic medium at room temperature with commercial 1-(2-methoxyphenyl) piperazine to obtain the respective 2-(4-(2-methoxyphenyl)piperazin-1-yl)-1-(1-(naphthalen-1-ylsulfonyl)-1*H*-indol-3-yl)ethan-1-one. Finally, the functionalized aminoketone obtained was reduced with sodium borohydride in methanol to yield the corresponding racemic alcohol (PUC-10).

2.3. Study of PUC-10 biopharmaceutical properties in silico

Physicochemical and drug-like properties for PUC-10 were calculated by SwissADME (Daina et al., 2017).

2.4. Preparation of lipid nanocapsules

Lipid nanocapsules (LNC) were prepared according to the temperature phase inversion method (Alvarez-Figueroa et al., 2022; Heurtault et al., 2002). The following components were added to a beaker: Lipoid P75®, Solutol HS 15®, NaCl, Labrafac PG® and Labrafil M 1944 CS®. This was left under magnetic stirring for 10 min and then heated up to 85 °C by a heating plate. Once this temperature was reached, the system was removed from the plate until it decreased to 55 °C. This cycle was repeated 3 times. After the third cycle, it was cooled to 70 °C and milli Q water was added at 0 °C, and it was left under magnetic stirring for 10 min. Finally, it was calibrated with milli Q water to a final volume of 2 mL.

Likewise, LNC loads with 300 μM PUC-10 (LNC-PUC-10) were made where the active ingredient was dissolved in one of the LNC components: Labrafac PG®. For this, enough PUC-10 was weighed on a microbalance (Sartorius micro, Fisons instruments, Italy) to obtain a 5 mg/mL solution of PUC-10 in Labrafac PG®. To ensure complete dissolution of the active ingredient, this was left under magnetic stirring for at least 12 h (PUC-10 in Labrafac PG®).

For to isolate LNC, they were filtered through a 0.22 μm filter.

2.5. Physicochemical characterization of the nanosystems

Photon correlation spectroscopy (PCS) was used to measure the hydrodynamic size of LNC and the polydispersity index (PI). For this, the samples were diluted in ultrapure water. Laser-Doppler was used to measure the zeta potential of these nanosystems (Zetasizer®, NanoZS, Malvern Instruments, Malvern, UK). In this case, the sample was diluted in 1 mM KCL. Finally, transmission electron microscopy (Inspect™, FEI Company, USA) was used to measure the morphology of the LNC. For

this, the samples were stained with 2 % phosphotungic acid.

2.6. Encapsulation and quantification of the 5-HT6 receptor antagonist (PUC-10)

To determine the PUC-10 encapsulation efficiency in LNC (E.E %), Eq. (1) was used. For this, it was necessary to quantify PUC-10 in non-isolated nanosystems and in isolated nanosystems. To break the nanosystems, 10 % SDS in acetonitrile was used.

$$E.E.(\%) = \frac{[\,isolated\ PUC - 10\ concentration^{*}100]}{[\,Non - isolated\ PUC - 10\ concentration]} \quad (1)$$

The quantification technique used was HPLC chromatography (HPLC Eksigent® Ekspert ™ UltraLC 100, AB Sciex, United States) which was coupled to a Tandem mass spectrometer (ABCsciex Triple Quad ™ 4500, AB Sciex, United States).

Chromatographic conditions were as follows: SunShell C8 column (2.6 μm; 4.6 × 150 mm, ChromaNik, Sweden), mobile phase acetonitrile: 0.1 % formic acid 70/30 with isocratic elution and at a flow rate of 1 mL /min. The total run was 3 min. The column temperature was maintained at 50 °C. The analysis software used corresponded to the Analyst 1.6.2 version.

The mass spectrometer was used maintaining a positive polarity. The optimized mass parameters were as follows: CUR: 20 Psi; IS: 4500v; Temp: 650 °C; GS1: 30 Psi; GS2: 40 Psi; DAC: 7.0v; DP: 176 v. The input potential energy was 10 V and the output potential energy was 18 V.

The PUC-10 calibration curve was prepared and measured each time the samples were analysed.

The concentrations of the calibration curve for each chromatographic analysis were 10, 25, 50, 100, 200 and 300 PPB, each sample being injected twice. It was sought that each time the calibration curve was made it had an R^2 value >0.99.

2.7. Stability of nanosystems

The stability of LNC and PUC-10-loaded nanosystems was analysed through their respective physicochemical characteristics (size, IPd and Z potential) when stored at a temperature of 4 °C. The measurement times were week 0 (Date of preparation), 1, 2, 3, 4, 8 and 12 weeks. The stability of the nanosystems incubated in DMEM (pH = 7.4) or diluted in water with 5 % DMSO, in both cases at a temperature of 37 °C, was also evaluated. In these cases, the measurements were made at 0, ¼, ½, ¾, 1, 2, 4, 6, 24 and 48 h.

In all cases, measurements were performed in triplicate.

2.8. Studies of cell viability

Cell viability studies were carried out on the SH-SY5Y cell line obtained from bone marrow neuroblastoma cultured in DMEM supplemented with fetal bovine serum (FBS) 10 % *v*/v and streptomycin-penicillin 1 % v/v at 37 °C in a 5 % CO_2 environment.

In a 96-well plate, 100,000 cells were seeded per well and left to incubate for 24 h, after which time the culture medium was removed from the wells and the treatment corresponding to 200 μL of each nanosystem was added.

After 24 h, 20 μL of Alamar Blue® reagent was added to each of the wells and allowed to incubate for 4 h, after which the fluorescence emitted by each well was measured using a plate reader (Cytation 5 Biotek, United States). Once with these data, we proceeded to calculate the % of cell viability using Eq. (2) (Carreño et al., 2022).

$$\%Cell\ viability = \frac{(\varepsilon OX)\lambda A2A\lambda 1 - (\varepsilon OX)\lambda 1A\lambda 2^{*}100}{(\varepsilon OX)\lambda 2A^{\circ}\lambda 1 - (\varepsilon OX)\lambda 1A^{\circ}\lambda 2} \quad (2)$$

Where:

λ1 = 570 nm, λ2 = 600 nm, (Ɛox)λ1 = Molar extinction coefficient for AlamarBlue at 570 nm: 80.586. (Ɛox)λ2 = Molar extinction coefficient for AlamarBlue at 600 nm: 17.216, Aλ1 = Treatment absorbance at 570 nm, Aλ2 = Treatment absorbance at 600 nm, A°λ1 = Negative control absorbance at 570 nm, A°λ2 = Negative control absorbance at 600 nm.

10 % DMEM was used as a negative control and Triton X-100 (1:10 in PBS) as a positive control.

To obtain the concentrations of each system, the mass of each component of the formulation was calculated, all masses were added and divided by the final volume of the formulation.

2.9. PAMPA-BBB experiments

The assay was performed in 96-well plates and the test was carried out from bottom to top. In the donor compartment was placed 330 μL of non-isolated LNC-PUC-10 diluted with 5 % DMSO (Bicker et al., 2016; Di et al., 2003; Wdowiak and Miklaszewski, 2023) (equivalent to 190.16 mg/mL of nanosystem and 180 μM of PUC-10). The donor compartment was covered with the plate corresponding to the receiving compartment which includes the membrane filters. Each filter was wetted with 4 μL of a 2 % *w/v* solution of porcine polar lipid in n-dodecane (Avdeef et al., 2007; Kato et al., 2023; Kim et al., 2022). In the receiving compartment was placed 330 μL of solution of a buffered sodium acetate: DMSO 95:5. Then the system was covered in the form of a "sandwich" and sealed with plastic and wet towels to prevent evaporation of the samples. Finally, the system was incubated for 18 h at 37 °C without agitation (Graverini et al., 2018; Sharma et al., 2019). After this time, the system was separated, and the acceptor solution was transferred to vials to quantify the PUC-10 by HPLC. Each experiment was performed in triplicate. As a positive control, thiopental was used at a concentration of 40 μg/mL (in a PBS, PBS: DMSO in a 95:5 ratio and solution sodium acetate buffer: DMSO in a 95:5 ratio).

To determine the permeability values, Eq. (3) was used (Balimane et al., 2005):

$$Pe = (V^{*}dC)/(A^{*}Co^{*}dT) \quad (3)$$

Where:

Pe = Permeability cm/s, V = Volume of the receiving well (cm3), A = Area of the well (0.3 cm^2 in this case), Co = Initial concentration in donor well (μM), dC/dT = Radius of change in concentration over time in acceptor well (μM/s).

3. Results and discussion

PUC-10, according to physicochemical properties obtained through the SwissADME web platform, has a high Log P (>4) and a Log S value less than −4, indicating its highly nonpolar character and poor water solubility (Daina et al., 2017; Daina and Zoete, 2016).

Some of these physicochemical properties are not optimal for PUC-10's passage through the blood-brain barrier. Studies have shown that key physicochemical properties enabling molecules to cross the blood-brain barrier include: a nitrogen and oxygen atom count sum of ≤5, a Log P between 1.5 and 2.7, a molecular weight less than 500 g/mol, and a number of aromatic rings between 1 and 4 (Rankovic, 2015; Wohlfart et al., 2012).

Evidently, PUC-10 does not meet all these properties.

Therefore, if we think about the nanoencapsulation of PUC-10, as it is a molecule that is poorly soluble in water, it is necessary to choose an oily excipient that can not only solubilize it but also not confer toxicity, so that it complies with GRAS (Generally Recognized as Safe) standards (Burdock and Carabin, 2004). GRAS compounds are listed under the Code of Federal Regulations (21 CFR) and many pharmaceutical excipients are part of this listing (Sosnik, 2013). Therefore, since each of the excipients used to make the nanocapsule has an adequate safety performance, it is guaranteed that both the nanosystems and their degradation products are also safe. Additionally, ideally the excipient

should have been used previously in the pharmaceutical industry (Heurtault et al., 2002; Kim et al., 2018; Ren et al., 2013). For this reason, Labrafac PG® was chosen as a solvent for PUC-10, which corresponds to a mixture of propylene glycol esters of caprylic acid (C8) and capric acid (C10), which is widely used as an oily vehicle in topical preparations and recurrently incorporated into lipid nanocapsules (Heurtault et al., 2002). Regarding the other components chosen for the LNC designed for this study we can point out that: i) Lecithin (Lipoid P75®) is a nonionic surfactant that corresponds to a mixture of phospholipids, fatty acids, and triglycerides, and has been reported to be essential to maintaining the stability of systems ranging from 50 to 100 nm (Minkov et al., 2005). ii) Solutol® HS 15 is a nonionic hydrophilic surfactant recognized for its high solubilizing power of lipophilic compounds. It is a mixture of PEG 660 12-hydroxystearate and free PEG 660 (Younes et al., 2018). iii) Labrafil® M 1944 CS is a non-ionic lipophilic surfactant that enhances penetration through mucous membranes, which is composed of a mixture of mono, di and triglycerides plus mono and di esters of long-chain fatty acids such as oleic acid and linoleic acid conjugated with PEG 300 (Li et al., 2017). It is expected that both Solutol® HS 15 and Labrafil M® 1944 CS would stabilize LNC by steric effect since both compounds have PEG groups (Heurtault et al., 2002; Huynh et al., 2009). The lipid nanocapsules' oily core is generally made up of medium to short chain fatty acids, stabilized by a mixture of lecithin and some pegylated surfactant (PEG) (Huynh et al., 2009). PEG is important since it has been studied that it temporarily inhibits the glycoprotein P (P-gp) activity of the cells that capture them, with and observed a higher concentration of nanoencapsulated drug inside them. Since there is a high expression of P-gp at the level of the central nervous system, these types of nanosystems facilitates the accumulation of the encapsulated drugs in the brain parenchyma (Moura et al., 2020).

The elaboration of the novel LNC was by means of an adaptation of the temperature phase inversion process (TIF), since it was worked with a reduction of 5 °C, to move away from the melting point of PUC-10 (89–90 °C) (Alvarez-Figueroa et al., 2022; Heurtault et al., 2002). The developed lipid nanocapsule is composed of an oily core composed of Labrafac PG® in which PUC-10 is dissolved. Lipoid P75®, Labrafil M 1944 CS® and and Solutol HS 15® are found surrounding this core, with the PEG chains facing outwards (see Fig. 2).

Thus, Table 1 shows the physicochemical characterization of the lipid nanocapsules (LNC) and the lipid nanocapsules loaded with the active compound (LNC-PUC-10). In both cases, the nanosystems were small (close to 50 nm), monodisperse (PI <0.3) and of potential Z negative close to −15 mV. It is also observed that these nanosystems were capable of efficiently encapsulating PUC-10.

Subsequently, the spherical shape of the LNC was verified by observation through scanning electron microscopy (STEM), see Fig. 3.

Regarding stability under storage conditions, it was shown that both nanocapsules, LNC and LNC-PUC-10, are stable for at least 4 weeks (see Fig. 4) maintaining their size and charge over time, without being influenced by the presence of an active molecule inside. Studies indicate that LCNs usually present high stability, possibly due to the presence of a hydrated layer formed by the solvation by the PEG groups of Solutol® HS 15 and Labrafil® M1944 CS, which covers the oily core and makes its aggregation difficult (Guerrini et al., 2018; Heurtault et al., 2002).

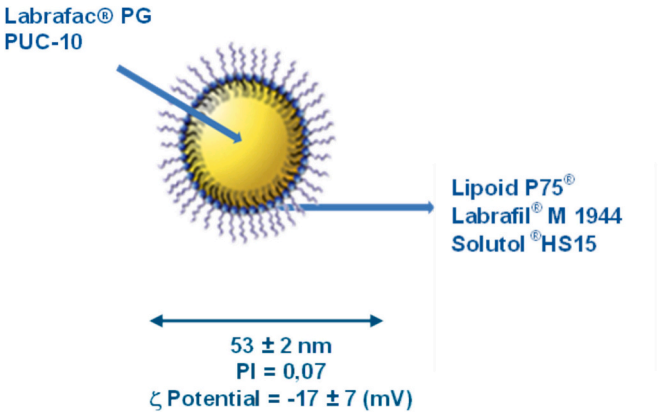

Fig. 2. Scheme of the new lipid nanocapsule designed to encapsulate PUC-10.

Table 1
Physicochemical characteristics of lipid nanocapsules.

Formulation	Size (nm)	PI	ζ Potential (mV)	% encapsulation
LNC	52 ± 3	0,129	−15 ± 1	
LNC-PUC-10	53 ± 2	0,093	−17 ± 7	74 ± 3

LNC: lipid nanocapsules. LNC-PUC-10: lipid nanocapsules loaded with PUC-10. $n = 3$.

Additionally, Fig. 5 shows that the nanocapsules at 37 °C in the different media studied maintain their physicochemical properties during the duration of the cell viability tests (4 h of contact with the nanosystems, DMEM pH 7.4) and PAMPA -BBB (18 h, water with 5 % DMSO). Furthermore, it was observed that in DMEM, the LNC-PUC-10 are more stable than the LNC, which would allow us to affirm that the incorporation of PUC-10 provides stability to the nanocapsules.

Next, the effect of the developed nanosystems on a cell line obtained from bone marrow neuroblastoma (SH-SY5Y) was studied. To carry out the Alamar Blue method, it was necessary to incubate different concentrations of the nanosystems on the cells for 4 h to subsequently determine the percentage of cell viability by absorbance. To determine the lipid nanocapsule concentration (mg/mL), all the components expressed in mass were added and divided by the final volume of the nanosystem. Through the cell viability data shown in Fig. 6, it is demonstrated how harmless these nanosystems are in all of the dilutions studied, indicating that they are safe and non-cytotoxic.

With the purpose of studying whether the developed LCN are effective as PUC-10 blood-brain barrier penetration promoters, an *in vitro* PAMPA-BBB permeability study was carried out. As mentioned above, PUC-10 presents physicochemical properties that are not favourable for crossing this barrier, such as its low aqueous solubility (log S < −6). In these tests, 5 % DMSO was used to solubilize PUC-10. This percentage of DMSO was chosen since higher concentrations, around 10 %, have been reported to exert an effect as penetration promoters, which would not allow us to observe the possible enhancer effect of the nanosystems (Kushwaha, 2018).

The conditions chosen for the PAMPA-BBB experiment (temperature, time, agitation) were validated by comparing the permeability of thiopental (positive control) dissolved in PBS with those reported in the literature ($P_{thiopental} = 18\,x10^{-6}$ cm/s) (Di et al., 2003). It was also verified, by calculating the permeability of thiopental, that the 5 % concentration of DMSO used did not exert a penetration-promoting effect (see Table 2).

There is a rule that establishes the requirements that a molecule must have to cross the blood-brain barrier: it must have high lipid solubility or a low capacity to form hydrogen bonds (that is, it must have less than 8 hydrogen bond donor or acceptor atoms) and a molecular weight less than 400 Da (Ghasemy et al., 2018; Pardridge, 2012). PUC-10 has a molecular weight greater than 400 Da so it does not comply with this dual rule, which predicts that PUC-10 would not permeate through this barrier. Additionally, the log *P* value of PUC-10 is quite high, which could allow this compound to interact with the membrane and be retained in it.

Thus, after carrying out the PAMPA-BBB study of PUC-10 dissolved in 5 % DMSO and diluted in sodium acetate buffer, as predicted, it failed to permeate the membrane and the calculated permeability was zero, which could be due to the phenomena previously explained. This demonstrates the need to study a system that promotes the penetration of PUC-10 such as its nanoencapsulation.

For its part, the nanoencapsulated PUC-10 managed to cross the

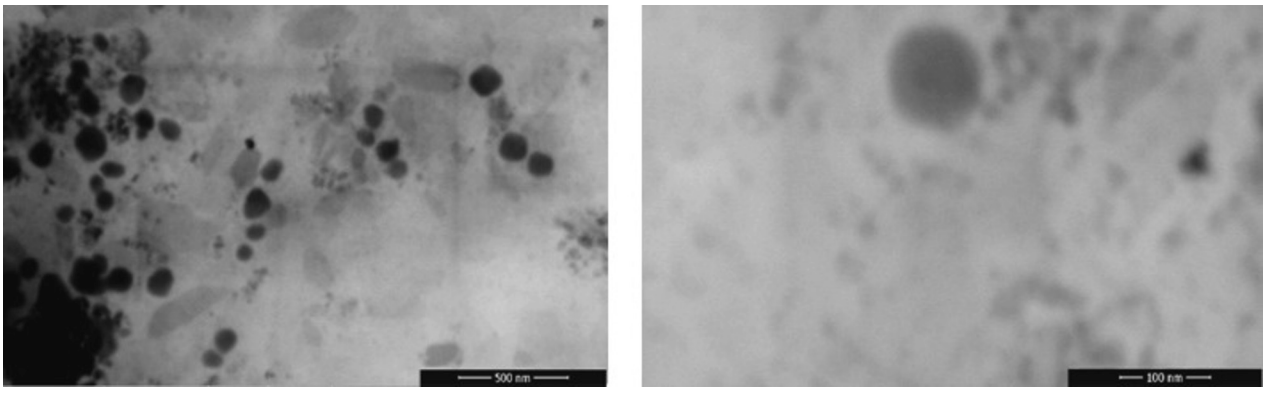

Fig. 3. Images obtained by scanning electron microscopy of the lipid nanocapsules loaded with PUC-10.

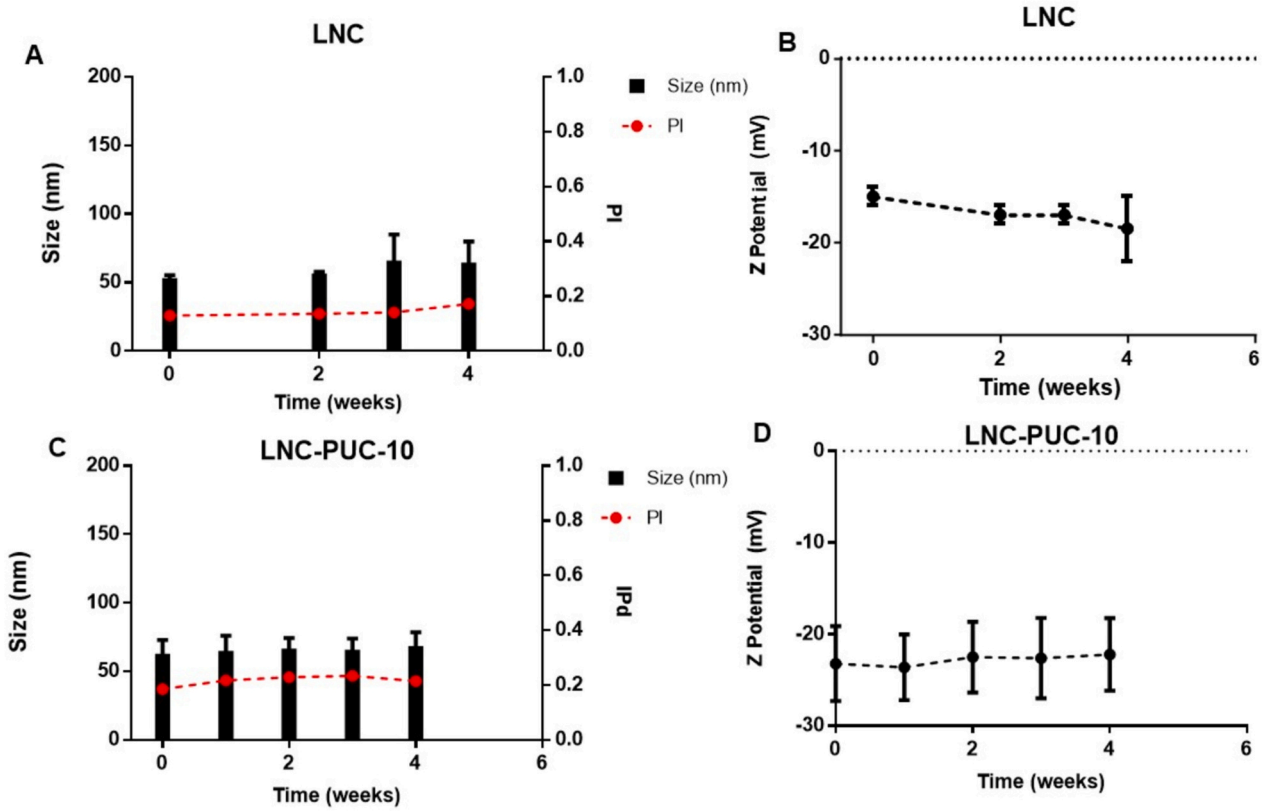

Fig. 4. Stability profile of of lipid nanocapsules in storage conditions (4 °C). A-C: Mean particle size. B-D: zeta potential. Mean ± S.D. (n = 3). A-B: lipid nanocapsules. C-D: lipid nanocapsules loaded with PUC-10.

simulated blood-brain barrier, finding permeability values of $P_{LNC\text{-}PUC\text{-}10} = 1,3\,x10^{-8}$ cm/s (see Table 2) (low permeability) (Di et al., 2003) demonstrating that nanoencapsulation promotes the passage of PUC-10 across the blood-brain barrier.

4. Conclusions

A novel lipid nanocapsule was designed and manufactured that efficiently encapsulates PUC-10, a novel N-Arylsulfonylindoles with a powerful antagonism on the 5-HT6 brain receptor, but that has physicochemical properties that make it difficult for it to pass through the blood-brain barrier. This nanosystem designed proved to be stable in different media and non-toxic (determined by cell viability assay). Finally, it was demonstrated, through PAMPA-BBB *in vitro* studies, that nanoencapsulated PUC-10 can cross the blood-brain barrier (permeability =1,3 * 10^{-8} cm/s) as opposed to unencapsulated PUC-10. With this, it is concluded that nanoencapsulating PUC-10 promotes its passage through the blood-brain barrier.

CRediT authorship contribution statement

María Javiera Alvarez-Figueroa: Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Francisco Nuñez-Navarro:** Data curation. **Gonzalo Recabarren-Gajardo:** Writing – review & editing, Investigation. **José Vicente González-Aramundiz:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization.

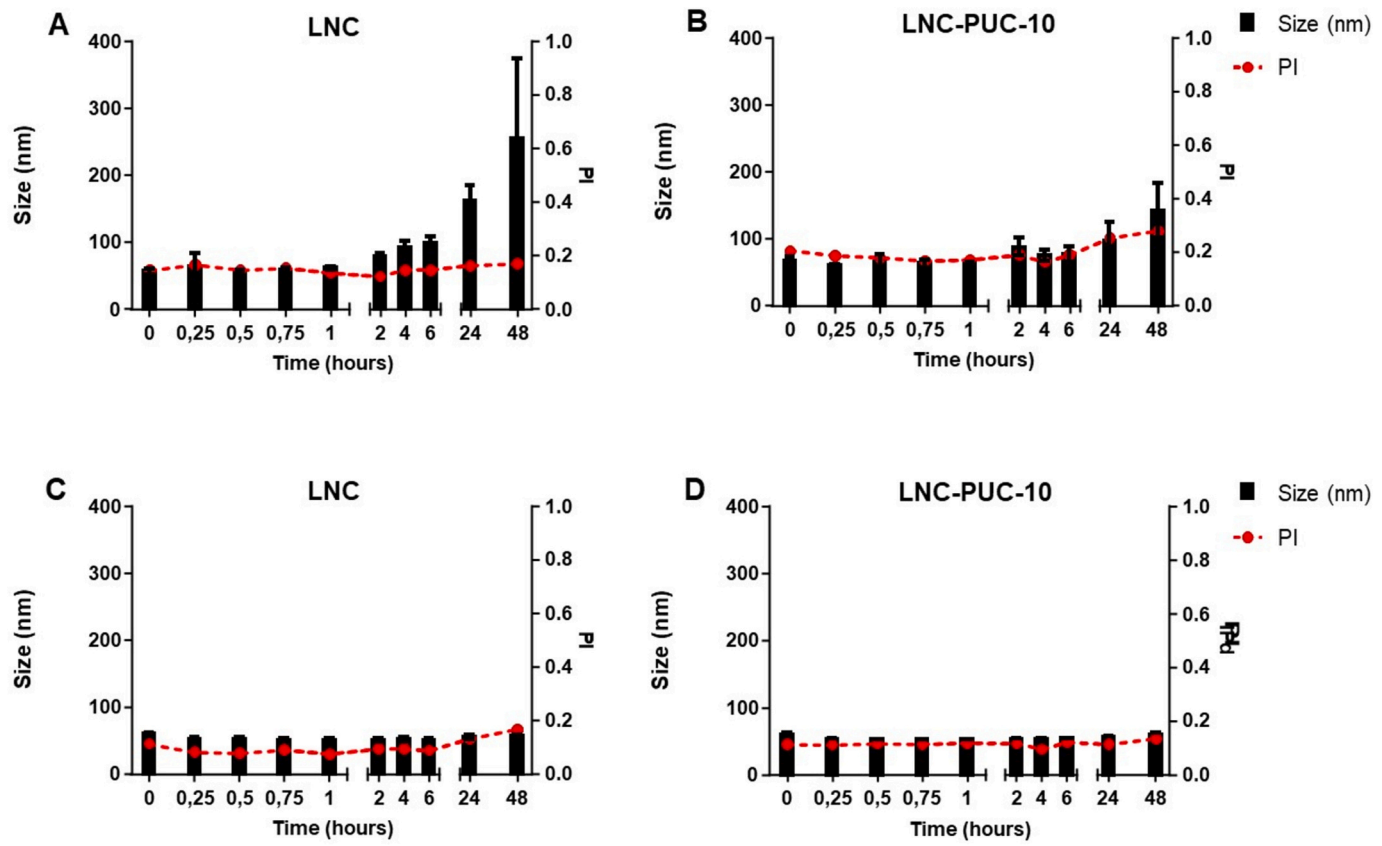

Fig. 5. Stability profile of lipid nanocapsules at 37 °C. A-B: Incubed in DMEM (pH 7.4). C-D: Incubed with 5 % DMSO. Mean ± S.D. (n = 3). LNC: lipid nanocapsules. LNC-PUC-10: lipid nanocapsules loaded with PUC-10.

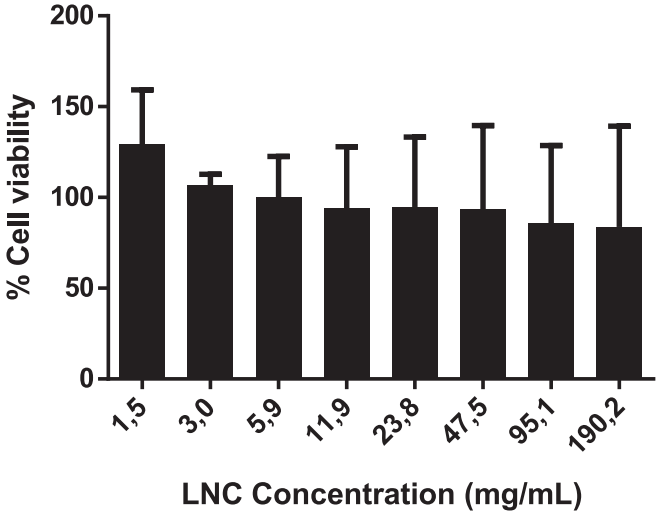

Fig. 6. Lipid nanocapsule cell viability (n = 3).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Maria Javiera Alvarez-Figueroa and Jose Vicente Gonalez-Aramundiz reports financial support was provided by Pontifical Catholic University of Chile. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 2
Thiopental and PUC-10 blood-brain barrier permeability by PAMPA-BBB.

Vehicle/ Solvent	Drug	Blood-brain barrier Permeability (cm/s)
PBS	Thiopental	$12{,}6*10^{-6}$
PBS with 5 % DMSO		$7{,}48*10^{-6}$
Acetate buffer (pH 5.5) with 5 % DMSO		$11{,}1*10^{-6}$
Acetate buffer (pH 5.5) with 5 % DMSO	PUC-10	0
Lipid Nanocapsules (LCN)		$1{,}3*10^{-8}$

PBS: Phosphate buffered saline; DMSO: dimethyl sulfoxide. LNC-PUC-10: nanocapsules loaded with PUC-10. n = 3.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by: Fondo Nacional de Desarrollo Científico y Tecnológico: Fondecyt Regular1240669, Fondecyt Regular 1241074, Fondo de Equipamiento Científico y Tecnológico: FONDEQUIP EQM130032 and EQM160042.

References

Alvarez-Figueroa, M.J., Alarcón, D.A., González-Aramúndiz, J.V., 2022. Effect of zeta potential of innovative lipid nanocapsules on triamcinolone transdermal delivery. Drug Deliv. Transl. Res. 12, 2740–2750. https://doi.org/10.1007/s13346-022-01134-5.

Arrieta-Rodríguez, L., Espinoza-Rosales, D., Vera, G., Cho, Y.H., Cabezas, D., Vásquez-Velásquez, D., 2021. Novel N-Arylsulfonylindoles Targeted as Ligands of the 5-HT

(6). In: Receptor. Insights on the Influence of C-5 Substitution on Ligand Affinity. Pharmaceuticals (Basel, Switzerland), p. 14. https://doi.org/10.3390/ph14060528.
Avdeef, A., Bendels, S., Di, L., Faller, B., Kansy, M., Sugano, K., Yamauchi, Y., 2007. PAMPA—critical factors for better predictions of absorption. J. Pharm. Sci. 96, 2893–2909. https://doi.org/10.1002/jps.21068.
Balimane, P.V., Pace, E., Chong, S., Zhu, M., Jemal, M., Pelt, C.K.V., 2005. A novel high-throughput automated chip-based nanoelectrospray tandem mass spectrometric method for PAMPA sample analysis. J. Pharm. Biomed. Anal. 39, 8–16. https://doi.org/10.1016/j.jpba.2005.03.043.
Bicker, J., Alves, G., Fortuna, A., Soares-da-Silva, P., Falcão, A., 2016. A new PAMPA model using an in-house brain lipid extract for screening the blood–brain barrier permeability of drug candidates. Int. J. Pharm. 501, 102–111. https://doi.org/10.1016/j.ijpharm.2016.01.074.
Burdock, G.A., Carabin, I.G., 2004. Generally recognized as safe (GRAS): history and description. Toxicol. Lett. 150, 3–18. https://doi.org/10.1016/j.toxlet.2003.07.004.
Carreño, G.F., Álvarez-Figueroa, M.J., González-Aramundiz, J.V., 2022. Dextran Nanocapsules with ω-3 in their Nucleus: an innovative Nanosystem for Imiquimod Transdermal delivery. Pharmaceutics 14, 2445.
Daina, A., Zoete, V., 2016. A BOILED-Egg to Predict Gastrointestinal Absorption and Brain Penetration of Small Molecules. Chemmedchem 11, 1117–1121. https://doi.org/10.1002/cmdc.201600182.
Daina, A., Michielin, O., Zoete, V., 2017. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci. Rep. 7, 42717. https://doi.org/10.1038/srep42717.
Di, L., Kerns, E.H., Fan, K., McConnell, O.J., Carter, G.T., 2003. High throughput artificial membrane permeability assay for blood–brain barrier. Eur. J. Med. Chem. 38, 223–232. https://doi.org/10.1016/S0223-5234(03)00012-6.
Fernandez, H.H., Weintraub, D., Macklin, E., Litvan, I., Schwarzschild, M.A., Eberling, J., Videnovic, A., Kenney, C.J., 2023. Safety, tolerability, and preliminary efficacy of SYN120, a dual 5-HT6/5-HT2A antagonist, for the treatment of Parkinson disease dementia: a randomized, controlled, proof-of-concept trial. Parkinsonism Relat. Disord. 114, 105511. https://doi.org/10.1016/j.parkreldis.2023.105511.
Ghasemy, S., García-Pindado, J., Aboutalebi, F., Dormiani, K., Teixidó, M., Malakoutikhah, M., 2018. Fine-tuning the physicochemical properties of peptide-based blood-brain barrier shuttles. Bioorg. Med. Chem. 26, 2099–2106. https://doi.org/10.1016/j.bmc.2018.03.009.
González-Vera, J.A., Medina, R.A., Martín-Fontecha, M., Gonzalez, A., de la Fuente, T., Vázquez-Villa, H., García-Cárceles, J., Botta, J., McCormick, P.J., Benhamú, B., Pardo, L., López-Rodríguez, M.L., 2017. A new serotonin 5-HT6 receptor antagonist with procognitive activity – Importance of a halogen bond interaction to stabilize the binding. Sci. Rep. 7, 41293. https://doi.org/10.1038/srep41293.
Graverini, G., Piazzini, V., Landucci, E., Pantano, D., Nardiello, P., Casamenti, F., Pellegrini-Giampietro, D.E., Bilia, A.R., Bergonzi, M.C., 2018. Solid lipid nanoparticles for delivery of andrographolide across the blood-brain barrier: in vitro and in vivo evaluation. Colloids Surf. B: Biointerfaces 161, 302–313. https://doi.org/10.1016/j.colsurfb.2017.10.062.
Grychowska, K., Satała, G., Kos, T., Partyka, A., Colacino, E., Chaumont-Dubel, S., Bantreil, X., Wesołowska, A., Pawłowski, M., Martinez, J., Marin, P., Subra, G., Bojarski, A.J., Lamaty, F., Popik, P., Zajdel, P., 2016. Novel 1H-Pyrrolo[3,2-c]quinoline based 5-HT6 Receptor Antagonists with potential Application for the Treatment of Cognitive Disorders Associated with Alzheimer's Disease. ACS Chem. Neurosci. 7, 972–983. https://doi.org/10.1021/acschemneuro.6b00090.
Guerrini, L., Alvarez-Puebla, R.A., Pazos-Perez, N., 2018. Surface Modifications of Nanoparticles for Stability in Biological Fluids, p. 11. https://doi.org/10.3390/ma11071154.
Heurtault, B., Saulnier, P., Pech, B., Proust, J.-E., Benoit, J.-P., 2002. A Novel phase Inversion-based Process for the Preparation of Lipid Nanocarriers. Pharm. Res. 19, 875–880. https://doi.org/10.1023/a:1016121319668.
Huynh, N.T., Passirani, C., Saulnier, P., Benoit, J.P., 2009. Lipid nanocapsules: a new platform for nanomedicine. Int. J. Pharm. 379, 201–209. https://doi.org/10.1016/j.ijpharm.2009.04.026.
Jaudoin, C., Agnely, F., Nguyen, Y., Ferrary, E., Bochot, A., 2021. Nanocarriers for drug delivery to the inner ear: Physicochemical key parameters, biodistribution, safety and efficacy. Int. J. Pharm. 592, 120038. https://doi.org/10.1016/j.ijpharm.2020.120038.
Jones, L.A., Sun, E.W., Martin, A.M., Keating, D.J., 2020. The ever-changing roles of serotonin. Int. J. Biochem. Cell Biol. 125, 105776. https://doi.org/10.1016/j.biocel.2020.105776.
Kato, R., Zeng, W., Siramshetty, V.B., Williams, J., Kabir, M., Hagen, N., Padilha, E.C., Wang, A.Q., Mathé, E.A., Xu, X., Shah, P., 2023. Development and validation of PAMPA-BBB QSAR model to predict brain penetration potential of novel drug candidates. Front. Pharmacol. 14, 1291246. https://doi.org/10.3389/fphar.2023.1291246.
Kim, D.H., Kim, J.Y., Kim, R.M., Maharjan, P., Ji, Y.-G., Jang, D.-J., Min, K.A., Koo, T.-S., Cho, K.H., 2018. Orlistat-loaded solid SNEDDS for the enhanced solubility, dissolution, and in vivo performance. Int. J. Nanomedicine 13, 7095–7106. https://doi.org/10.2147/IJN.S181175.
Kim, C.K., Ahn, J., Yu, J., Le, D., Han, S., Lee, M., 2022. Analysis of Antioxidant Constituents from Ilex rotunda and Evaluation of their Blood-Brain Barrier Permeability. Antioxidants (Basel, Switzerland) 11. https://doi.org/10.3390/antiox11101989.
Kushwaha, A., 2018. A Screening of Permeation Enhancers for Transdermal delivery of Propofol. J. Bioequivalen. & Bioavailabilit. 10. https://doi.org/10.4172/0975-0851.1000378.
Lacivita, E., Perrone, R., Margari, L., Leopoldo, M., 2017. Targets for Drug Therapy for Autism Spectrum Disorder: challenges and Future Directions. J. Med. Chem. 60, 9114–9141. https://doi.org/10.1021/acs.jmedchem.7b00965.
Li, Y.J., Hu, X.B., Lu, X.L., Liao, D.H., Tang, T.T., Wu, J.Y., Xiang, D.X., 2017. Nanoemulsion-based delivery system for enhanced oral bioavailability and caco-2 cell monolayers permeability of berberine hydrochloride. Drug Deliv. 24, 1868–1873. https://doi.org/10.1080/10717544.2017.1410257.
Li, X., Gao, L., Liu, J., Zhang, H., Chen, H., Yang, L., Wu, M., Li, C., Zhu, X., Ding, Y., Sun, L., 2021. Safety, Tolerability and Pharmacokinetics of the Serotonin 5-HT6 Receptor Antagonist, HEC30654, in healthy Chinese Subjects. Front. Pharmacol. 12, 726536. https://doi.org/10.3389/fphar.2021.726536.
Minkov, I., Ivanova, T., Panaiotov, I., Proust, J., Saulnier, P., 2005. Reorganization of lipid nanocapsules at air–water interface: I. Kinetics of surface film formation. Colloids Surf. B: Biointerfaces 45, 14–23. https://doi.org/10.1016/j.colsurfb.2005.03.009.
Monsma Jr., F.J., Shen, Y., Ward, R.P., Hamblin, M.W., Sibley, D.R., 1993. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. Mol. Pharmacol. 43, 320–327.
Moura, R.P., Pacheco, C., Pêgo, A.P., des Rieux, A., Sarmento, B., 2020. Lipid nanocapsules to enhance drug bioavailability to the central nervous system. J. Control. Releas. : Off. J. Controll. Release Soc. 322, 390–400. https://doi.org/10.1016/j.jconrel.2020.03.042.
Pajouhesh, H., Lenz, G.R., 2005. Medicinal chemical properties of successful central nervous system drugs. NeuroRx : J. American Soci. Experiment. NeuroTherapeut. 2, 541–553. https://doi.org/10.1602/neurorx.2.4.541.
Pardridge, W.M., 2012. Drug transport across the blood-brain barrier. J. Cereb. Blood Flow Metab. 32, 1959–1972. https://doi.org/10.1038/jcbfm.2012.126.
Pateiro, M., Gómez, B., Munekata, P.E.S., Barba, F.J., Putnik, P., Kovacevic, D.B., Lorenzo, J.M., 2021. Nanoencapsulation of Promising Bioactive Compounds to Improve their Absorption, Stability, Functionality and the Appearance of the Final Food Products. Molecules 26. https://doi.org/10.3390/molecules26061547.
Ramírez, M.J., 2013. 5-HT6 receptors and Alzheimer's disease. Alzheimers Res. Ther. 5, 15. https://doi.org/10.1186/alzrt169.
Rankovic, Z., 2015. CNS drug design: balancing physicochemical properties for optimal brain exposure. J. Med. Chem. 58, 2584–2608. https://doi.org/10.1021/jm501535r.
Ren, S., Mu, H., Alchaer, F., Chtatou, A., Müllertz, A., 2013. Optimization of self nanoemulsifying drug delivery system for poorly water-soluble drug using response surface methodology. Drug Dev. Ind. Pharm. 39, 799–806. https://doi.org/10.3109/03639045.2012.710634.
Rolley, N., Bonnin, M., Lefebvre, G., Verron, S., Bargiel, S., Robert, L., Riou, J., Simonsson, C., Bizien, T., Gimel, J.C., Benoit, J.P., Brotons, G., Calvignac, B., 2021. Galenic Lab-on-a-Chip concept for lipid nanocapsules production. Nanoscale 13, 11899–11912. https://doi.org/10.1039/d1nr00879j.
Sharma, P., Tripathi, A., Tripathi, P.N., Prajapati, S.K., Seth, A., Tripathi, M.K., Srivastava, P., Tiwari, V., Krishnamurthy, S., Shrivastava, S.K., 2019. Design and development of multitarget-directed N-Benzylpiperidine analogs as potential candidates for the treatment of Alzheimer's disease. Eur. J. Med. Chem. 167, 510–524. https://doi.org/10.1016/j.ejmech.2019.02.030.
Sosnik, A., 2013. Reversal of multidrug resistance by the inhibition of ATP-binding cassette pumps employing "generally Recognized as Safe" (GRAS) nanopharmaceuticals: a review. Adv. Drug Deliv. Rev. 65, 1828–1851. https://doi.org/10.1016/j.addr.2013.09.002.
Valdivia-Olivares, R.Y., Martinez-González, E.A., Montenegro, G., Bridi, R., Alvarez-Figueroa, M.J., González-Aramundiz, J.V., 2023. Innovative multiple nanoemulsion (W/O/W) based on Chilean honeybee pollen improves their permeability, antioxidant and antibacterial activity. Food Res. Int. (Ottawa, Ont.) 168, 112767. https://doi.org/10.1016/j.foodres.2023.112767.
Vera, G., Lagos, C.F., Almendras, S., Hebel, D., Flores, F., Valle-Corvalán, G., Pessoa-Mahana, C.D., Mella-Raipán, J., Montecinos, R., Recabarren-Gajardo, G., 2016. Extended N-Arylsulfonylindoles as 5-HT$_6$ Receptor Antagonists: Design, Synthesis & Biological Evaluation. Molecules 21. https://doi.org/10.3390/molecules21081070.
Wdowiak, K., Miklaszewski, A., 2023. Amorphous System of Hesperetin and Piperine-Improvement of Apparent Solubility. Permeabili.,Biol. Activit. 24. https://doi.org/10.3390/ijms24054859.
Williams, J., Siramshetty, V., Nguyễn, Đ.T., Padilha, E.C., Kabir, M., Yu, K.R., Wang, A. Q., Zhao, T., Itkin, M., Shinn, P., Mathé, E.A., Xu, X., Shah, P., 2022. Using in vitro ADME data for lead compound selection: an emphasis on PAMPA pH 5 permeability and oral bioavailability. Bioorg. Med. Chem. 56, 116588. https://doi.org/10.1016/j.bmc.2021.116588.
Wohlfart, S., Gelperina, S., Kreuter, J., 2012. Transport of drugs across the blood-brain barrier by nanoparticles. J. Control. Releas. : Off. J. Controll. Release Soc. 161, 264–273. https://doi.org/10.1016/j.jconrel.2011.08.017.
Younes, N.F., Abdel-Halim, S.A., Elassasy, A.I., 2018. Solutol HS15 based binary mixed micelles with penetration enhancers for augmented corneal delivery of sertaconazole nitrate: optimization, in vitro, ex vivo and in vivo characterization. Drug Deliv. 25, 1706–1717. https://doi.org/10.1080/10717544.2018.1497107.